ENDOCRINOLOGY AND METABOLISM

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A History of Metabolism

GRAHAM LUSK

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Introduction

When one considers the history of the development of the science of nutrition one is impressed with the gradual growth of knowledge upon the subject. The ideas concerning it are not the products of the work of supermen. The ideas were not born as was Minerva, who sprang from the head of Jove. And yet those who furthered science were men possessing much information and also a sense of appreciation of values.

"Not from a vain or shallow thought
His awful Jove young Phidias brought."

Though vain and shallow men may contribute for weal or woe to political or social life, they have no influence upon science.

This history has been composed with the dominant viewpoint of presenting the subject in the words of the Old Masters themselves. One would not desire to see an imitation of the Sistine Chapel could one view the reality itself.

The Dawn of History

It is interesting to note that Voit (*d*) attributes the higher cultivation of the peoples living in the temperate zones to the distribution of food. He says in this regard:

"The ingestion of food is a fundamental condition of our existence and is indeed one of the most wonderful arrangements of Providence. To the blinded eyes of man it often appears as a punishment that by the sweat of his brow he should eat bread. Hunger is the primary and most powerful spur to work, and only through work come experience and progress. If we were provided with sufficient available energy for life we would ever remain in an undeveloped state. In a country where nature with outstretched arms offers excess of nourishment which is obtainable without effort, one will seek in vain for independent, driving progress. Originally, prehistoric man was nomadic, living temporarily

upon the country where he settled. He tamed wild animals for his service. He then drifted into the most fruitful land areas and these he cultivated. Here came the dawn of history.

"In the tropics the development of man is prevented by an enervating atmosphere. In the polar regions where the greatest exertion results in obtaining only a small amount of sustenance progress is also limited. Eskimo and Lapp live as they did a thousand years ago and have no history. In temperate climes the production of food is not so favored as in warmer regions, but the other conditions for the maintenance of an active life are more favorable and therefore civilization will ever have her home there."

The Classical Period

The Greeks had no classical education but it has been said that they had the two essential requisites of true education, the capacity to express themselves in words and a desire to understand their relations with their environment, of which the latter is science (Prof. E. H. Starling). Epictetus makes the statement and gives the advice which follows: "Socrates in this way became perfect, in all things improving himself, attending to nothing except to reason, but you who are not yet a Socrates ought to live as one who wishes to be a Socrates." This was the general attitude of the scholars of Greece and Rome.

Socrates (*B. C. 470-399*) held that the object of food was to replace the loss of water from the skin and the loss of ponderable heat.

Hippocrates (*B. C. 460-364*), the Father of Medicine and a contemporary of Socrates, believed that the loss of body weight in fasting was due to the loss of "insensible perspiration" from the skin and to a loss of heat which he conceived to consist of a fine material. Among the writings of Hippocrates may be found the following aphorisms:

Aphorism, Sec. I, 14.—Growing bodies have the most innate heat; they therefore require the most food, for otherwise their bodies are wasted. In old persons the heat is feeble and therefore they require little fuel as it were to the flame, for it would be extinguished by much. On this account, also, fevers in old persons are not equally acute, because their bodies are cold.

Aphorism 4, Sec. II.—Neither repletion nor fasting nor anything else is good when more than natural.

Aphorism 38.—An article of food or drink which is slightly worse but more palatable is to be preferred to such as are better but less palatable.

The Greeks believed that there were four elements, fire, air, earth and water, and four elemental properties, hot, cold, moist and dry. The broad viewpoint of Hippocrates thus finds expression:

Whoever having undertaken to speak and write on medicine have first laid down for themselves some hypothesis to their argument such as hot or cold or

moist or dry or whatever else they choose (thus reducing their subject within a narrow compass and supposing only one or two original causes of disease or of death among mankind) are clearly mistaken in much that they say.

Aristotle (*B. C. 384-322*) created the conception of a functioning organism in the following celebrated passage:

> The animal organism is to be conceived after the similitude of a well governed commonwealth. When order is once established in it there is no more need of a separate monarch to preside over each separate task. The individuals each play their assigned part as it is ordered, and one thing follows another in its accustomed order. So in animals there is the same orderliness—nature taking the place of custom—and each part naturally doing his own work as nature has composed them. There is no need of a soul in each part, but she resides in a kind of central governing place in the body and the remaining parts live by continuity of natural structure and play the parts nature would have them play.

Galen (*A. D. 131-200*), a physician from Troy who practiced in Rome six hundred years after Socrates, was unable to add anything to the ancient doctrines taught by the Greeks. Galen remarks, "The blood is like the oil, the heart is like the wick and the breathing lungs an instrument which conveys external motion."

The Dark Ages

For thirteen hundred years after the time of Galen knowledge of nutrition did not advance. The alchemists were at work striving to make gold from the baser metals and endeavoring to produce infallible medicines. But in the absence of a knowledge of the chemistry of living things there could be no knowledge of the function of food.

Carl Voit(*d*), possibly with a slight national bias, thus portrays the events in the dark ages:

> One usually regards this period of the world as intellectually barren, during which only a blind imitation of the old and senseless scholasticism prevailed. However, one makes a great mistake to condemn the human race as having been incapable for a thousand years. We should rather understand why a rapid development was impossible. The conditions for a continued expansion of scientific knowledge were about as unfavorable as imaginable. The Age of Antiquity reached the highest standard of cultivation possible from the knowledge of the time and it needed entirely new ideas in order to move forward, for the cultivation of mankind is not accomplished like a constantly growing branch, but rather like one which is stimulated anew after having been formerly ripe. I doubt whether the ancient Greeks and Romans with their peculiar mental temperament had the power further to extend knowledge. The Empires in which the old cultivation had flourished went down, and younger races reigned in their stead. These rough victors eagerly acquired the intellectual treasures which the conquered people had accumulated in the days of their glory; they regarded themselves as pupils and fell for a time into intellectual dependence

as they devoutly entered into this great heritage. The education of peoples is like that of an individual. It is some time after education in the schools has taught one to think that one is capable of independent action, and usually one seeks first the wrong way before one finds the right. Even so, the change from the olden to the modern could take place only after prolonged struggle. The spirit was gradually sharpened but there were not enough new facts to create new ideas. Satisfaction was sought in acute dialectics. This was only an indication that the old methods brought no one forward. Finally, the tremendous events which took place in the fifteenth century changed dutiful scholars into critics and independent investigators who, through the revelation of heretofore unknown methods of the mind, were able to open up new pathways.

The Renaissance

The universities of Cambridge (founded in 1229) and Oxford (founded in 1249) were established at a time when authority was worshiped. After the revival of learning in Italy the original versions of the ancient classics were brought into France and England and the forgotten culture of a bygone civilization was revived.

The Greek idea of medicine persisted after two thousand years and Chaucer (1340-1400) portrays the physician as follows:

> "He knew the cause of every malady,
> Were it of cold or hot or moist or dry,
> And where engendered and of what humour,
> He was a very perfect practisour."

No adequate conception of the nature of nutrition was possible without an understanding of the nature of air. The idea that air was an elementary substance persisted until comparatively recent times. The groping of human inquiry into the analysis of the invisible atmosphere constitutes a fascinating chapter.

Leonardo da Vinci (1452-1519), accounted one of the greatest painters of the Renaissance and who was at the same time mathematician, physicist and naturalist, said at the end of the fifteenth century that no animal, whether of the land or of the air, could live in an atmosphere which could not support a flame (Milne-Edwards I, 377). The broad mind of Leonardo with wonderful intuition interprets life as follows:

> Hast thou marked Nature's diligence? The body of everything that takes nourishment constantly dies and is constantly reborn; because nourishment can only enter into places where that past nourishment has expired, and if it has expired it has no more life; and if you do not supply nourishment equal to the nourishment departed life will fail in vigor; and if you take away this nourishment life is utterly destroyed. But if you restore as much as is consumed day by day, just so much of life is reborn as is consumed; as the flame of the candle is fed by the nourishment given by the liquor of the candle, which flame continually with rapid succor restores from below what above is consumed in

dying; and from a brilliant light is converted into dark smoke; which death is continuous as the smoke is continuous; and the continuance of the smoke equals the continued nutriment; and at the same moment all the flame is dead and regenerated with the movement of its nutriment.

Paracelsus (1493-1591) recognized the analogy between the production of heat without flame, both in the body and chemically outside the body, as had Aristotle and Galen before him. He imagined the existence of a spirit, the *Archæus,* which lived in the stomach and which there divided the foods into the good and the bad, the former being used by the body and the latter being eliminated in the excreta as evil and poisonous.

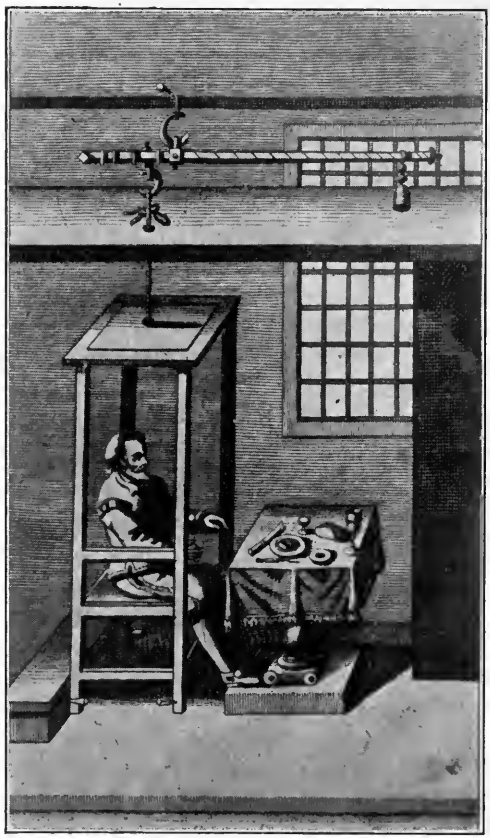


Fig. 1. Frontispiece of "De medicina statica aphorismi," showing Sanctorius seated on a chair suspended from a large steelyard.

Sanctorius (1561-1636), a professor of Padua, published in 1614 his celebrated "De medicina statica aphorismi," which was printed in Venice. Sanctorius kept careful account of his body weight, noted also the weight of food and drink taken and of urine and excrement passed. He was thus able to discover that the major evacuation from the body was the "insensible perspiration." He determined the considerable loss in body weight during periods in which no urine or feces were passed from the body. Section III of the Aphorisms treats "of Meats and Drink" and contains the following quaint allusions, as rendered in a translation by John Quincy, published in London in 1712 and printed for William Newton in Little Britain.

LXXV. "The Physician who has the Care of the Health of *Princes* and and knows not what they daily perspire, deceives them and will never be able to cure them except by Accident."

LXXVI. "In the first four Hours after Eating a great many perspire a Pound or near; and after that to the ninth two Pound; and from the ninth to the sixteenth scarce a Pound."

VIII. "Mutton easily digests and perspires; or it will waste in a night a third part of a Pound more than any other usual Food."

XXIII. "Pork and Mushrooms are bad both because they do not Perspire themselves and because they hinder the Perspiration of other things eat along with them."

LIX. "If a Supper of eight Pounds corrupts in the Stomach, the next Day the Body will be lighter than after a Supper of three Pound which does not do so."

These aphorisms summarized signify that a well appreciated meat, such as mutton, increases the perspiration, whereas pork, which very likely then as now was an unpopular food in Italy, causes "corruption" and diarrhea and hence no increase in perspiration.

This kind of investigation was continued by Dodart (died 1707) in France, who devoted thirty-three years of exhaustive labor to the subject.

Then followed the first discovery of carbonic acid gas by Van Helmont in the seventeenth century.

Van Helmont (1577-1644), a member of the ancient princely family of the Counts of Mérode of Belgium, was one who consecrated his life to his laboratory. He discovered that when charcoal burned or wine fermented a gas was produced which was as invisible as respired air; that it is sometimes emitted from the bowels of the earth in mines or at the Grotto del Carno (near Naples—so called because if a man enters it accompanied by a dog, the man lives but the dog dies, since carbon dioxid gas evolved is heavier than air and remains near the ground); that it is present in the waters of Spa and is evolved when vinegar is poured on chalk. This *gaz sylvestre* ("wood gas") does not maintain a flame nor life of animals. It promptly results in their asphyxiation and death.

Jean Rey, born about the end of the sixteenth century, died 1645, a physician of Périgord, found in 1630 that tin and lead increased in weight when calcined, but the significance of these facts was neglected in the subsequent enthusiasm over phlogiston. Rey's work, "Essays sur la recherche de la cause pour laquelle l'éstain et le plomb augmentent de poids quand on les calcine," 1630, was reprinted after Lavoisier's discoveries in 1777.

Nicholas Lefevre (died 1674), in his "Traité de Chimie," published about 1660, says, "In the act of respiration the air does not confine itself to refreshing the lungs, but by means of the 'universal spirit' it reacts upon the blood, refining it and volatilizing all its superfluities." A hundred years later Haller had about the same viewpoint. Lefèvre was one of the founders of the Académie des Sciences and was physician-in-chief to Louis XIV.

Robert Boyle (1621-1679) in 1660 showed that the flame of a candle or the life of an animal was extinguished after placing them in an air pump. Between 1668 and 1678 he made numerous experiments with

many animals of different species with a view of isolating that part of the air which was "eminently respirable." Thus he suggests in a subdivision entitled "Of Air in Reference to Fire and Flame" in his work on "The General History of the Air" (1680) the following experiments:

The burning of candles under a glass bell.

The burning of spirits of wine under a glass bell.

The keeping of animals in the same instrument whilst the flame is burning.

In the "Sceptical Chemist," which appeared in 1661, Boyle thus voices his opinions:

> Now a man need not be very conversant in the writings of chemists to observe in how lax, indefinite and almost arbitrary senses they employ the terms *salt, sulphur* and *mercury*. . . .
>
> But I will not here enlarge upon this subject nor yet will I trouble you with what I have largely discoursed in the "Sceptical Chemist," to call in question the grounds on which chemists assert that all mixed bodies are compounded of salt, sulphur and mercury.

Boyle lived in the period of the birth of national scientific societies. The Académie des Sciences was founded in Paris by Louis XIV, who, after the peace of the Pyrenees in the fullness of his power, felt that his kingdom needed nothing further than to be fortified by science, industry and art, and he instructed his minister Colbert to carry out his desires. The members were given stipends from the state. This was the first example of state endowment of science. About the same time the Royal Society of London was established in England, which was the outgrowth of a gathering of men at first held surreptitiously. This older organization, of which Boyle was a member, is still perpetuated as the Royal Society Club.

Among those influenced by Boyle was one John Mayow.

John Mayow (1640-1679), "descended from a genteel family of his name living at Bree in Cornwall, was born in the parish of St. Dunstan-in-the-West, in Fleet Street, London, admitted as a scholar of Wadham College the 23rd of September, 1659, aged sixteen years," (Beddoes). His scientific work was accomplished at All Soul's, Oxford. Some of his experiments may be thus recounted:

Camphor placed in a capacious glass vessel inverted over water is ignited by a burning glass. After cooling, the air is reduced one-thirtieth in bulk. A second piece of camphor will not burn, "a clear proof that the combustion has deprived the air of its fire-air particles so as to have rendered it altogether unfit to support flame."

A mouse was put into a wire trap and this was placed on a three-legged stool which stood in water and the whole was covered with a bell jar. The volume of the air diminished one-fourteenth.

If a burning candle and an animal be put together in a bell jar both will go out sooner than one alone because flame is extinguished and an animal expires for want of nitro-aërial particles.

"Air loses somewhat of its elastic force during its respiration by animals, as also in combustion. One must believe that animals, like fire, remove from air particles of the same nature."

And in another place he writes, "Breathing brings the air into contact with the blood to which it gives up its nitro-aërial constituent. Again the motion (of the muscles) results from the chemical action in the muscle with the combustible matter contained therein."

Niter contains the nitro-aërial particles and hence gunpowder burns without air. Many authors have written "as if it had been ordained that *niter* should make as much noise in philosophy as in war, yet its properties are still concealed from our knowledge."

Calcined antimony mixed with niter, when acted on by heat from a burning glass, increases in weight through addition of nitro-aërial particles.

As to Mayow's death, at the age of thirty-nine it was written:

"He paid his last debt to nature in an apothecary's house bearing the sign of the Anchor in York Street near Covent-Garden, within the liberty of Westminster (having been married a little before not altogether to his content), in the month of September, 1679, and was buried in the Church of St. Paul, Covent-Garden."

Beddoes, his biographer, writes: "Mayow . . . silently and unperceived in the obscurity of the last century discovered if not the whole sum and substance, yet certainly many of those splendid truths which adorn the writings of Priestley, Scheele, Lavoisier, Crawford, Goodwyn and other philosophers of this day."

"Should I ask you who of all your acquaintance is the person least likely to be overtaken by surprise you would, I think, name a certain Northern Professor. . . . Yet at the sight of the annexed representation of Mayow's pneumatic apparatus, this sedate philosopher lifted up his hands in compleat astonishment."

The "sedate philosopher" was undoubtedly Black. Writing in 1790, however, Beddoes cannot escape from the absurd statement, "He (Mayow) has clearly presented the notion of phlogiston which rendered the name of Stahl so celebrated."

Mayow's "Treatise on Respiration" was published in his twenty-eighth year. Newton invented the calculus when twenty years old; Black found "fixed air" at twenty-four; R. Mayer formulated the Law of the Conservation of Energy at twenty-six.

Mayer's paper containing the last-named doctrine was refused publication in Liebig's *Annalen!* These facts should afford a stimulus to the young and food for the thought of the more mature.

Willis (1621-1675), a contemporary of Boyle, and his pupil Lower, a colleague of Mayow at Oxford, demonstrated the reddening of blood by the respiration by admitting and excluding air from an animal.

Stephen Hales (1677-1761) was a parish priest described by Horace Walpole as "a poor, good, primitive creature." And yet this apparently unimportant man writes in his "Statical Essays," published in 1727, "A part of the inspired air is lost in the blood, but it is as yet entirely dark what its use may be."

Boerhaave (1668-1738), when he published his great work, the "Elements of Chemistry," in 1724, is believed to have had the work of Mayow in mind when he wrote: "Who can say whether an air of special virtue for the maintenance of the lives of animals and plants does not exist; whether it may not become exhausted; whether its consumption is not the cause of the death of animals who can no longer possess it? Many chemists have announced the existence of a vital element in the air, but they have never told what it is or how it acts. Happy the man who discovers it!"

Stahl (1660-1734), the German chemist who in 1716 moved to Berlin as physician to the King of Prussia, was the originator of the *phlogiston* theory of combustion which enthralled the minds of men for nearly a hundred years. According to this theory all combustible substances contained phlogiston which passed from them when they were burned. What we now know as oxids of iron or lead were those metals which through burning had lost their phlogiston. Such substances, if calcined with carbon, a material supposed to be rich in phlogiston, absorbed phlogiston and became metals once more. This simple theory availed to explain all the phenomena of combustion and was generally accepted by the scientific world.

When one halts to consider the general knowledge of nutrition in the middle of the eighteenth century one finds little to distinguish between the statements of Sanctorius, 150 years earlier, and Benjamin Franklin. Sanctorius writes, "Meats which promote Perspiration bring Joy, but those which obstruct it Sorrow"; and Franklin in 1742, "If thou art dull and heavy after Meat it is a sign that thou hast exceeded due measure; for Meat and Drink ought to refresh the Body and make it cheerful and not to dull or oppress it."

The general opinion of high authorities in the eighteenth century was voiced by Haller.

Albrecht von Haller (1708-1777), the great physiologist, published his "Elementa Physiologica" between 1757 and 1765. He asserts "that fire is contained in the blood is proved by its heat," and he has this rather hazy conception of the process of respiration: "The secondary uses of respiration are very numerous. It exhales copiously and removes from the blood something highly noxious; for by remaining in the air

it will cause suffocation; and the breath of many people crowded in a close and small place impregnates the air with a suffocating quality. On the other hand, it absorbs from the air a thin vapor, of which the use is not sufficiently known."

And Benjamin Franklin in "Poor Richard," 1746, thus poetically popularizes the ideas of his time:

"Like cats in air pumps to subsist we strive,
On joys too thin to keep the soul alive."

The dawn of the modern era has been reached, but there is little to indicate the impending clarification of thought. Before considering the events which led to the Chemical Revolution one must stop to learn of a case of self-inflicted human scurvy.

William Stark, M.D. (1740-1770).—The work of Stark was edited after his death by J. C. Smyth.

In the editor's preface one reads, "His experiments on diet are the first and will probably long remain the only experiments of the kind."

It is stated that he began his experiments on diet in 1769, greatly encouraged by Dr. Franklin, "from whom he received many hints."

Stark thus describes himself: "The person upon whom these experiments are tried is a healthy man about twenty-nine years of age, six feet high, stoutly made but not corpulent, of a florid complexion, with red hair."

He reached the following general conclusions: "A very spare and simple diet has commonly been recommended as most conducive to health, but it would be more beneficial to mankind if we could shew them that a pleasant and varied diet was equally consistent with health as the very strict regimen of Cornaro or the Miller of Essex. These and other abstemious people, who having experienced the great extremities of bad health, were driven to temperance as their last resource, may run out in praises of a simple diet, but the probability is that nothing but the dread of former sufferings could have given them resolution to persevere in so strict a course of abstinence."

He gives the following reasons for undertaking the investigation: "Dr. B. Franklin of Philadelphia informed me that he himself when a journeyman printer lived a fortnight on bread and water at the rate of ten pounds of bread per week and found himself stout and hearty on this diet." . . .

"I learned from Dr. Mackenzie that many of the poor people near Inverness never took any kind of animal food, not even eggs, cheese, butter or milk."

Mr. Hewson told him that a ship's crew, having consumed the provisions, lived one part on tobacco, the other part on sugar. The latter

generally died of scurvy, while the former remained free from the disease or soon recovered.

Dr. Cirelli informed him that Neapolitan physicians frequently gave for periods of forty days no food to patients suffering from fever.

Mr. Slingsby has lived many years on bread, milk and vegetables without animal food or wine and has been free from gout ever since he began this regimen.

Stark's experiments of taking bread and water alone may thus be summarized:

	Daily diet oz.	Body weight at start lbs.	Period
Period I	Bread, 20	171	2 weeks
" II	Bread, 30	163	3 "
" III	Bread, 30	161	5 days
" IV	Bread, 38	158	1 week
		160 (at end)	

"During the third period I was one day irregular, having ate about four ounces of meat and drank two or three glasses of wine. At the conclusion of it I was perfectly hearty, my head clear, often hungry."

After this, from July 26 to August 24, he took a diet of bread, water and sugar. On August 11, "I now perceived small ulcers on the inside of my cheeks, particularly near a bad tooth; the gums of the upper jaw of the same side were swelled and red and bled when pressed with the finger; the right nostril was also internally red or purple and very painful."

On August 13, having been extremely ill, he took a few ounces of meat and two or three glasses of wine with his bread. This caused marked improvement in his condition. On August 22 he dined heartily on meat and fruit and drank some wine.

From August 24 to September 13, a diet of bread, water and olive oil. On September 8 he was so weak that he almost fainted when walking across the floor. The gums were swollen and he "spat in considerable quantity a very disagreeable, fetid, yellowish fluid." On September 9 he took "a basin of mutton broth" and thereafter lived freely on animal food, milk and wine until September 18, when "I felt myself quite recovered."

On September 18 to October 2, a diet of bread, water and milk. Upon this diet the gums improved and the offensive smell disappeared.

From October 2 to October 14 the diet consisted of bread, water and roast goose. He became "hearty and vigorous, both in mind and body."

October 14 to 19 lived freely on animal food.

October 21 to 28, bread, water and boiled beef. "Never the least heavy or dull, . . . but had a keenness for study."

October 28 to November 1, diet of bread, water and sugar. The gums were not affected by the sugar.

November 17 to 20, lean beef, 20 oz. Upon this diet he felt hungry.

November 21 to 25, lean beef, 20 oz., and suet, 7 oz. "I slept longer and more quietly than formerly and was more disposed to be drowsy than when I lived on meat alone."

November 26 to December 8, flour, 20 oz.; suet, 4 to 6 oz. This diet was arranged in order to compare its value with that of meat. It was taken in the form of a pudding. He notes an extraordinary gain in body weight of 8 lbs., in five days after changing the dietary from meat to flour, (*vide* later experiments of Voit, p. 70).

December 9 to 13, flour, 24 oz. Upon this diet he became extremely hungry.

He finds that flour and beef suet disagree with him, tries to substitute butter fat for beef suet, but does not return to a normal appetite until he has enjoyed eating two pounds of figs. In another experiment he has indigestion after taking for four days puddings made of flour and butter.

February 4 to 15. Bread and flour with honey. Scorbutic symptoms developed on February 12. Honey pudding had a remarkable diuretic effect and provoked diarrhea.

On February 15 he was feeble and took an infusion of rosemary.

February 16 and 17. Diet—bread with Cheshire cheese to check the diarrhea, which it did.

February 18 he omits cheese but continues with the infusion of rosemary. His mouth is sore, there are pimples at the corner of his mouth and many large ones on his body.

This closes his diary.

On February 18 he was bled, but died on February 23, 1770, evidently of acute intestinal infection, the victim of his scientific curiosity. John Hunter made a report of the findings at the autopsy.

The Chemical Revolution

Out of the misty conclusions of the middle of the eighteenth century before its close modern chemistry developed. The work of Mayow was forgotten in the enthusiasm over the phlogiston doctrine of Stahl. The pioneer discoverer was again an Englishman, Joseph Black. It is quite probable that had Mayow known of Black's "fixed air" he might have solved the problem of respiration. And also had Black known of the existence of Mayow's experiments without having learned of them to his

"compleat astonishment", he too might have had the honor reserved for Lavoisier.

Black (1728-1799) in 1754 published a Latin essay which, in its English form, is entitled "Experiments on Magnesia Alba, Quicklime and other Alkaline Substances." In this Black describes the discovery of "fixed air" or carbonic acid. Black writes of himself as follows:

> In the early days of my chymical studies the author whose works made the most agreeable impression on my mind was Markgraaf (1709-1782) of Berlin; he contrived and executed his experiments with so much chymical skill that they were uncommonly instructive and satisfactory; and he described them with so much modesty and simplicity, avoiding entirely the parade of erudition and self-importance, with which many other authors encumber their works, that I was quite charmed with Markgraaf and said to Dr. Cullen that I would rather be the author of Markgraaf's Essays than of all the chymical works in the library. The celebrated Reaumur's method of writing appeared to me also uncommonly pleasing. After three years spent with Dr. Cullen I came to Edinburgh to finish my education in medicine. Here I attended the lectures of Dr. Monroe, senior, and the other medical professors until the summer of 1754 when I received the degree of Doctor of Medicine and printed my inaugural dissertation, "De Humore Acido à Cibis Orto, et Magnesia Alba."

Black finds that the carbonates yield "fixed air" on ignition and that caustic alkalis absorb the same air. Magnesia alba loses half its weight when heated and gives off "fixed air" when treated with acids. Lime water does not combine with ordinary air but does combine with "fixed air." Black describes the new found kind of air as one "which is dispersed through the atmosphere either in the state of a very subtle powder, or more probably in that of an elastic fluid. To this I have given the name of *fixed air,* and perhaps very improperly; but I thought it better to use a word already familiar in philosophy than to invent a new name, before we are more fully acquainted with the nature and properties of this substance."

This was the pioneer discovery in the field long known as pneumatic chemistry. "Fixed air" was produced in fermentation, in the combustion of carbon, and was eliminated in the respiration. The next gas to be discovered was hydrogen.

Cavendish (1731-1810) was a nephew of the third Duke of Devonshire. He was a man of wealth and of extremely eccentric character. It was he who discovered hydrogen in 1766 and gave it the name of "inflammable air." He considered hydrogen to be phlogiston. Later, in 1781, he found that when two volumes of "inflammable air" and one volume of Priestley's "dephlogisticated air" (oxygen) were united by an electric spark the airs disappeared and water resulted. Cavendish concluded that dephlogisticated air was water deprived of its phlogiston.

The French have always claimed that Lavoisier was the first to dis-

cover the composition of water. A discussion of the Water Controversy is given by Thorpe.

Daniel Rutherford (1749-1819) was a pupil of Black's and the uncle of Sir Walter Scott. Rutherford in 1772 described "a residual air," or nitrogen gas, as it is now called. He found that when a candle burned in an inclosed place until it went out and the "fixed air" was then absorbed by alkali, there remained a large volume of air which extinguished life and flame in an instant.

Fig. 2. Priestley. From an engraving of a portrait by Gilbert Stuart.

Priestley (1733-1804) in 1771, a year before Rutherford's discovery of nitrogen, introduced a growing sprig of mint into an atmosphere in which a candle had burned out and after a lapse of several days found that another candle burned in it perfectly. Evidently the burning candle filled the space with phlogiston; the growing plant absorbed the phlogiston and produced "dephlogisticated air." This could again receive phlogiston when the second candle burned.

Shortly after this discovery (1774) Priestley submitted red oxid of mercury to the heat of a burning glass and found that an air was evolved in which a candle burned very vigorously. Priestley assumed that this air was pure dephlogisticated air, while common air was only partly dephlogisticated.

And Priestley writes, "My reader will not wonder that, after having ascertained the superior goodness of dephlogisticated air by mice living in it and the other tests above mentioned, I should have the curiosity to taste it myself. I have gratified that curiosity by breathing it, drawing it through a glass siphon, and by this means I reduced a large jar full of it to the standard of common air. The feeling of it to my lungs was not sensibly different from that of common air; but I fancied that my breath felt peculiarly light and easy for some time afterward. Who can tell but that in time this pure air may become a fashionable article in luxury? Hitherto only two mice and myself have had the privilege of breathing it."

Priestley explained the presence of Black's "fixed air" in the ex-

pired air thus: "It will follow that in the precipitation of lime by breathing into lime water the fixed air which incorporates with lime comes not from the lungs but from the common air, decomposed by the phlogiston exhaled from them." And Priestley, who was one of the discoverers of oxygen, was gathered to his fathers at Northumberland, Pennsylvania, in 1804, still believing the phlogiston theory of combustion.

Crawford (1748-1795) was the first individual to publish experiments on animal calorimetry. In 1777 he found, after burning wax and carbon or on leaving a live guinea-pig in his water calorimeter, that for every 100 oz. of oxygen used the water was raised the following number of degrees Fahrenheit:

Wax	2.1
Carbon	1.93
Guinea-pig	1.73

Crawford states, "Animal heat seems to depend upon a process similar to a chemical elective attraction." However, the theory of phlogiston renders Crawford's work quite unintelligible and in the second edition of his "Experiments and Observations—Animal Heat," published in 1788, one still finds statements like this, "Now it has been proved that when an animal is surrounded by a medium at a low temperature it phlogisticates a greater quantity of air in a given time than when it is surrounded by a warm medium."

Scheele (1742-1786).—Independent of Priestley and before him, Scheele, a Swedish apothecary and eminent chemist, discovered oxygen by decomposing dioxid of manganese and other substances. Scheele believed that the atmosphere was composed of "spoiled air" and "fire air." When a body burned in air it lost its phlogiston, which united with "fire air." Heat consisted of "fire air" united with phlogiston. It passed through glass. In this way a portion of air could pass through glass.

In 1771 Scheele (Scheele, 1793) had found that when silver carbonate was heated in a retort "fixed air" was liberated as well as "fire air," while a residue of metallic silver remained. In 1775 he placed silver carbonate in a small retort connected with a collapsed bladder and then heated the substance. Two airs were evolved, "fixed air" which he removed with lime water, and "fire air" in which a flame burned brightly. In the interim between these two experiments he wrote Lavoisier in Paris a letter dated September 30, 1774, asking him to use his powerful burning glass upon silver carbonate, then to absorb the "fixed air" in lime water and observe whether a candle would burn and an animal live in the remaining air, and he begged Lavoisier to inform him of the results.

Scheele performed another striking experiment (Scheele, 1777). He placed two large bees together with a little honey in a small upper chamber

of a glass apparatus which he had devised. This upper chamber was in communication with a glass cylinder. The glass cylinder he filled with "fire air" and immersed its lower end in lime water. The volume of the air within the receptacle diminished day by day and the lime water which absorbed the carbonic acid rose in the tube. After eight days the bees were both dead and the lime water almost completely filled the space.

Fig. 3. Scheele's apparatus showing bees in the upper chamber of a glass apparatus filled with oxygen.

It is evident that Scheele had introduced bees into pure or nearly pure oxygen gas and that the carbon dioxid which they produced had been completely absorbed by the lime water.

Scheele made no direct comment upon this truly beautiful experiment but in the general criticism of several experiments one may read the following hazy generalization:

> Why do not the blood and lungs change "fire air" into "acid air"? I take the liberty to express my opinion concerning this, for what would such exacting experiments profit unless through them I had the hope to more nearly approach my ultimate aim, the truth. Phlogiston, which combines with most substances causing them to become more fluid, more mobile and more elastic, must have the same influence upon the blood. The blood corpuscles must absorb it from the air through delicate openings in the lungs. Through this combination they are expanded and in consequence become more fluid. In some part of the circulation they must give off this absorbed phlogiston and consequently be able to again absorb this fine principle when they next reach the lungs. Whither the phlogiston goes during the circulation I will leave to others to find out. The affinity of blood for phlogiston cannot be as great as in the instance of plants and insects which take it from the air and also the blood cannot convert it into "acid air," but it is changed into a kind of air which is midway between "fire air" and "acid air"; it is "spoiled air." For it does not unite with lime water or water as does "fire air," though it extinguishes fire as does "acid air."

Scheele's "spoiled air" was nitrogen. The poor struggling apothecary who had made so many careful and accurate experiments and who was one of the greatest chemists of his time, was unable to interpret his results without adherence to the dominant fetish of phlogiston.

We have here the picture of two earnest men, Priestley and Scheele, both absorbingly interested in chemistry, both contributing important knowledge and ranking among the greatest scientists of their day, and yet neither had the philosophical acumen to understand the meaning of his experiments. Priestley was a Dissenting clergyman, earning his living by preaching, but in his old age his house was burned by Loyalists and he

shortly afterward fled to America. Scheele, though honored by scientific men the world over, remained a poor apothecary to the end of his days. In the current parlance of to-day these two great contributors to human knowledge would undoubtedly have been known outside their own circles as "narrow-minded scientists."

This, however, could never have been said of Lavoisier, who repeated and extended their experiments, overthrew the phlogiston theory and established modern chemistry.

Lavoisier (1743-1794).—The family of Antoine Laurent Lavoisier traced its ancestry back seven generations to Antoine Lavoisier, who was a post-boy in the stables of the king and who died in 1620. Successive generations raised the position of the family name to ever higher levels until it was said of the great Lavoisier that it would require perhaps a hundred years for the appearance of his equal. Native intelligence, a fine education, great wealth, combined with the environment of the searchingly critical atmosphere of the Paris of his day, allowed of the vivid inspiration which filled his life.

Lavoisier was elected a member of the Académie des Sciences in 1768 at the age of twenty-four. About the same time, desirous of promoting his personal fortune, he became associated with *la ferme générale,* through whose activities the taxes were collected in France. Some of his fellow academicians looked askance at this undertaking, but the mathematician Fontaine is reported to have remarked, "Never mind, he will be able to give us better dinners." (Grimaux, (*k*) 1896.)

In the *ferme générale* the young man was the subordinate of one Paulze, a nephew of the then all-powerful Terray, Minister of State and Controller of Finance. At the age of twenty-eight Lavoisier married the fourteen-year-old daughter of Paulze. His own position and his marriage brought him great wealth but in no way diminished his tireless activity. He congratulated himself that his patronage of the instrument makers of Paris had rendered France independent of Great Britain in the manufacture of scientific instruments.

Lavoisier's first paper before the Académie was "On the Nature of Water and on Those Experiments Which Pretend to Prove Its Transformation Into Earth." In this experiment he placed rain water in a flask and boiled it for 101 days. Mineral matter appeared in the flask but the whole did not change in weight and the mineral material which appeared was shown to be derived from the disintegration of the flask itself, which lost in weight. Lavoisier used an extremely sensitive (*très exacte*) balance, made by the official who was charged with the weighing of gold.

Here we witness the overthrow of a dogma more than two thousand years old, accomplished by the introduction of the quantitative method into

chemistry. One may recall the words of Lavoisier written in his "Elements of Chemistry" (Robert Kerr, (*m*) 1799):

As the usefulness and accuracy of chemistry depend entirely upon the determination of the weights of the ingredients and products both before and after experiments, too much precision cannot be employed in this part of the subject and for this purpose we must be provided with good instruments. . . . I have three sets (of balances) of different sizes made by M. Fontin with the utmost nicety; and excepting those made by Mr. Ramsden of London I do not think that any compare with them in precision and sensibility.

Fig. 4. Lavoisier and his wife. From an engraving of a portrait by David.

Lavoisier had a balance which could weigh 600 gm. within five mg. and another which was sensitive to within a tenth of a milligram, which were quite up to modern standards of accuracy. One may visit the Conservatoire des Arts et Métiers in Paris and see there a notable collection of Lavoisier's apparatus. One sees a gasometer for the accurate measurement of gases; there is the celebrated ice calorimeter of Lavoisier and La Place; there also are barometers of finest workmanship, set in mahogany supports decorated with gilded filagree work, reminding one of the choicest furniture. These treasures were placed in the cellar of the Conservatoire during the bombardment of Paris by the Germans in the late war.

Concerning the gasometers, Lavoisier wrote (Lavoisier, (*m*) 1799):

It becomes expensive because in many experiments, such as the formation of water and of nitric acid, it is absolutely necessary to employ two of the same machines. In the present advanced state of chemistry very expensive and complicated instruments are become indispensably necessary for ascertaining the analysis and synthesis of bodies with the requisite precision as to quantity and proportion.

It is strange that Lavoisier's insistence upon the use of accurate, quantitative measurements through the application of which nearly a hundred and fifty years ago he brought about the "Chemical Revolution," should appear as new truth when enunciated by some of our ultra modern scientists.

In the heart of France near Puy-du-Dom, at Château de la Carrière, now owned by Monsieur de Chazelles, there is a veritable museum of scientific apparatus which formerly belonged to Lavoisier (Truchot, (*s*) 1879). There are several thermometers of great accuracy and a fine

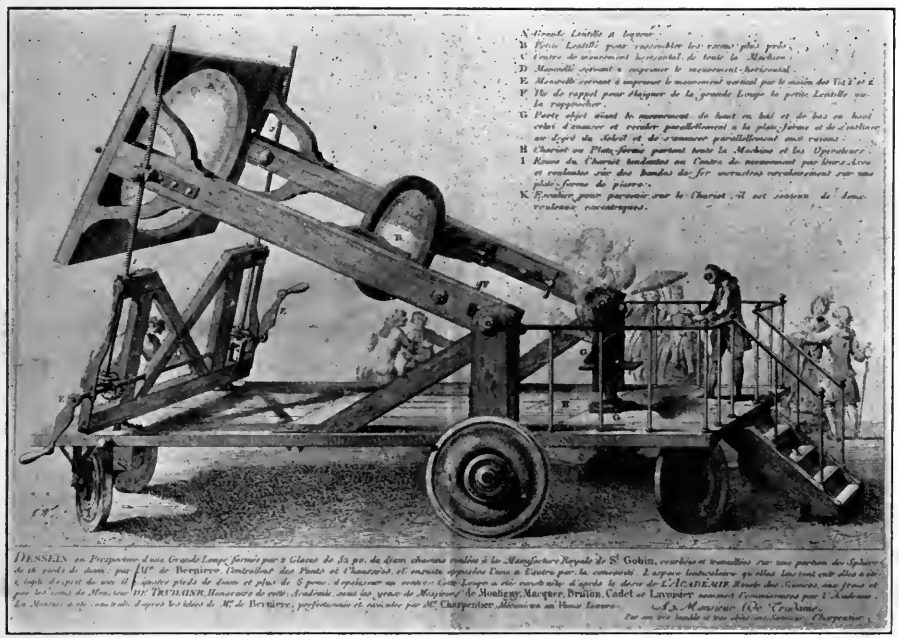


Fig. 5. The burning glass of Trudaine. From "Œuvres de Lavoisier," Vol. III, Pl. IX.

balance, and there are three large glass globes, one capable of holding 15 liters of air, another 12 liters and a third 7 liters; also many another treasure of great historic value. Lavoisier made his experiments before the days when rubber and cork reduced laboratory expenses. His glass tubes and receptacles were united with finely polished brass joints.

We may imagine this accomplished Frenchman at work in his laboratory, or his library, or receiving information from visitors to the fashionable and brilliant capital of France. It is related (Thorpe, (*r*) 1908) that Priestley dined with Lavoisier in Paris in October, 1774, and informed him concerning the production of "pure dephlogisticated air" from oxid of mercury, and we may also recall that Scheele, on September 30 of the same year, wrote to Lavoisier, asking him to expose silver carbonate to

the heat rays of a large burning glass and produce "fixed air" and "fire air" from them. Ten days after his conversation with Priestley, and again during the month of the following March, Lavoisier went to Montigny to visit his friend Trudaine, who was the owner of an immense burning glass 42 ins. in diameter, which had cost 15,000 livres (about $3,000), and he here repeated Priestley's experiments. In the paper read before the Académie des Sciences at Easter, 1775, Lavoisier (*a*) stated that he took the red mercury calx and heated it with carbon and obtained "fixed air," and when he heated the same without carbon a gas was evolved in which a flame burned with the splendor of phosphorus in air, and that this gas was the "air eminently respirable." The loss in weight of the mercury calx was equal to the weight of the "air eminently respirable" given off. He concluded that "fixed air" was the result of the union of carbon with "air eminently respirable." In a subsequent paper he reported that it was this "air eminently respirable" which was absorbed by phosphorus and sulphur when they burn with the production of phosphoric and sulphuric acids (*b*).

Having discovered these facts, Lavoisier (*c*) proceeded to determine the effect of a sparrow upon the content of air in a confined space. In a brief memoir published in 1777 he enunciated the principles that during respiration it was only "air eminently respirable" (oxygen) which disappeared from the atmosphere when an animal was put into a confined space and that this air was supplanted by expired "aëriform calcic acid" (carbon dioxid); that when metals were calcined in air oxygen was absorbed until its supply was exhausted; that if after an animal had perished in a confined space and the carbon dioxid in the atmosphere was absorbed by alkali the "foul air" remaining was the same kind of air as that found after metals had been calcined in air in an inclosed space. All the former qualities of this air could be restored by adding to it "air eminently respirable."

Three years later Lavoisier and La Place made another step in advance. (Lavoisier and La Place, (*n*) 1780.) They noticed that a guinea-pig produced 224 grains of carbonic acid in ten hours, and that what would now be called the *respiratory quotient* was 0.84. Then they put another guinea-pig in their recently constructed ice calorimeter and found that the heat given off by the animal melted 13 oz. of ice in a period of 10 hours. Next they calculated that if carbon was oxidized so that 224 grains of carbonic acid were produced, 10.4 oz. of ice would have been melted. They realized that in the case of the guinea-pig allowances would have to be made (1) because the legs of the animal became chilled during the experiment; (2) because the water of respiration was added to that of the melted ice; and (3) because the influence of cold increased the heat production of the animal. But they nevertheless stated that "Since we have found in the preceding experiments that the two qualities of heat

obtained are nearly the same, we can conclude directly and without hypothesis that the conservation of animal heat in the animal body is due, at least in greater part, to the transformation of 'air pur' (oxygen) into 'air fixe' (carbonic acid) during the process of respiration." Here be it noted that Lavoisier refers to the *conservation of animal heat* more than fifty years before the general law of the conservation of energy was enunciated. He also observed that two sparrows produced about the same quantity of carbonic acid in the unit of time as did a guinea-pig.

About a year after these experiments (1781) Cavendish in England found that when "inflammable air" (or hydrogen) and Priestley's "dephlogisticated air" were united by an electric spark the airs disappeared and water resulted.

It is said that Lavoisier, hearing of these experiments from Blagden, secretary of the Royal Society of London, repeated them. But the important point is that Lavoisier (*d*) was the first really to understand the phenomenon. In a memoir presented to the Académie des Sciences in 1783 he stated that water is merely a combination of "inflammable air" and oxygen and that any heat or light produced by their union is imponderable.

In the same year Lavoisier (*e*) completely demolished the phlogiston hypothesis and concluded his memoir "Reflections upon Phlogiston" with these words:

> My object in preparing this memoir has been to record the new developments of the theory of combustion which I published in 1777, to show that the phlogiston of Stahl, which he gratuitously supposed existed in metals, sulphur, phosphorus and all combustible substances, is an imaginary creation. All the phenomena of combustion and calcination are much more readily explained without phlogiston than with phlogiston. I understand that my ideas will not be suddenly adopted. The human mind conforms to a certain manner of vision and those who during a portion of their lives comprehend nature from a given point of view have difficulty in acquiring new ideas. In good time the opinions I have set forth will be confirmed or destroyed. In the interim, it is a great satisfaction for me to see that young, unprejudiced minds among those who are commencing to study science, such as mathematicians and physicists who have a new sense of chemical truths, no longer believe in phlogiston as presented by Stahl but regard the whole doctrine as scaffolding which is more embarrassing than it is useful for the continuance of the structure of the science of chemistry.

And the wonder of it all is that the great chemists of his time outside of his own country persisted in their narrow viewpoint. Priestley and Cavendish refused to be converted. Scheele wrote in 1783, "Is it impossible to convince Lavoisier that his system will not find universal acceptance? The idea of nitric acid from nitrous air and pure air, of carbonic acid from carbon and pure air, of sulphuric acid from sulphur and pure air, of lactic acid from sugar and pure air!! Can one believe such things? Rather will I support the English."

Only Black, professor of chemistry at Edinburgh and the discoverer of "fixed air," saw the truth. Lavoisier wrote to Black on November 13, 1790, a letter (Richet, (*p*) 1887) composed six months after the reading of his last memoir to the Académie des Sciences. He concluded the letter with the truest French courtesy: "It is only right that you should be the first to be informed of progress in a field which you opened and in which we all regard ourselves as your disciples. We do the same kind of experiments and I have the honour to communicate to you the results of our recent discoveries. I have the honour to remain, with respectful attachment, etc."

And to this Black replied in 1791, "The numerous experiments which you have made on a large scale and which you have so well devised have been persued with so much care and with such scrupulous attention to details that nothing can be more satisfactory than the proofs you have obtained. The system which you have based on the facts is so intimately connected with them, is so simple and so intelligible, that it must become more and more generally approved and adopted by a great number of chemists who have long been accustomed to the old system. . . . Having for thirty years believed and taught the doctrine of phlogiston as it was understood before the discovery of your system, I for a long time felt inimical to the new system which represented as absurd that which I had hitherto regarded as sound doctrine, but this enmity which springs only from force of habit has gradually diminished, subdued by the clearness of your proofs and the soundness of your plan."

In reading of the overthrow of the old doctrine of the fire principle phlogiston one must feel a throb of the impending horror of the French Revolution when one considers the statements of Marat written in 1791. Marat at one time had declared that a flame, when placed in a confined vessel, went out because the heat of the flame suddenly expanded the air, causing such a pressure on the flame that it was extinguished. Lavoisier refuted this doctrine. Marat, "L'Ami du People," under the title "Modern Charlatans," published the following: "Lavoisier, the putative father of all the discoveries that are noised about, having no ideas of his own, snatches at those of others, but having no ability to appreciate them, he quickly abandons them and changes his theories as he does his shoes." Certainly words of unqualified, contemporaneous disapproval!

Lavoisier's new system of salts and oxids led him to forecast the discovery of sodium and potassium, for in his "Elements of Chemistry" (Lavoisier, (*m*) 1799) he wrote, "It is quite possible that all the substances we call earths may be only metallic oxids irreducible by any hitherto known process." A eulogist of Lavoisier has likened this to the vision of Copernicus before Galileo's invention of the telescope.

Lavoisier had now progressed so that he was able to lay the fundamental basis of modern chemical physiology. Thus, in 1785, he stated

that the discrepancy between the quantity of expired carbonic acid and inspired oxygen, which he had observed in 1780, was accounted for by the fact that a part of the absorbed oxygen was utilized to oxidize hydrogen in the lungs. This oxidation would produce additional heat and account for the discrepancy between the heat directly measured from a guinea-pig and the heat calculated as being derivable from the oxidation of carbon by oxygen. It is interesting to recall that eighty years later, in 1860, Bischoff and Voit still calculated the heat value of the metabolism from the heat which would be produced in burning the carbon and hydrogen elements of the metabolism.

Respiration experiments on a human being constituted the final contribution in the culmination of this great career. The work is presented by Seguin and Lavoisier (*t*) in the memoirs of the Académie des Sciences during the year 1789. In this paper the authors remark: "This analogy between combustion and respiration did not escape the attention of the poets and philosophers of antiquity, of which they were the interpreters and spokesmen. Fire taken from the heavens, this flame of Prometheus, not only represents an idea that is ingenious and poetical but it is a true picture of the operations of nature on behalf of animals who respire; one can say with the ancients that the fire is lighted the moment a baby takes its first respiration and is not extinguished until its death."

Before giving the details of the experiments on man the authors state that a guinea-pig respired in pure oxygen and in a mixture of oxygen and hydrogen gas just as it did in ordinary air; respiration, circulation and the intensity of combustion were uninfluenced. Nitrogen had nothing to do with respiration.

In the experiments on man Seguin himself was the subject. The results are given in the accompanying table:

RESULTS OF EXPERIMENTS ON MAN

Condition	Environmental Temperature Degrees	Oxygen Absorbed per Hour	
		Pouces	Liters
(1) Without food	26	1210	24
(2) Without food	12	1344	27
(3) With food		1800-1900	38
(4) Work (9,195 foot pounds) without food		3200	65
(5) Work (9,750 foot pounds) with food		4600	91

Here are the basic facts regarding metabolism. The *basal metabolism* was increased 10 per cent after exposure to cold; 50 per cent after taking food; 200 per cent by exercise; and 300 per cent on combining the influences of food and exercise. We now know more details and we may also calculate that Lavoisier's determination of 24 liters of oxygen absorbed

per hour in this first historical experiment on the basal metabolism was 25 per cent too high. As for the experimental plan, it is as modern as the work of to-day, and yet it was executed 140 years ago by the first man who really understood the significance of oxygen. It is only in the last decade that the summation of the individual stimuli caused by food and muscular work and noted by Lavoisier has been verified. Lavoisier (*p*). also observed a constant relation between the quantity of oxygen consumed and the rate of the pulse multiplied by the number of respirations.

How Lavoisier achieved these remarkable results is not known, for the times in which he lived became too troubled to allow further work in pure science. We find, however, the following statement in the original memoir: "It would have been impossible to accomplish these exact experiments upon respiration before the introduction of a simple, easy and rapid method of gas analysis. This service M. Seguin has rendered to chemistry."

If, now, one turns to the report of Seguin (Seguin (*q*), 1791) one finds that he states that in his work with Lavoisier he used eudiometers 8 to 10 inches high and an inch in diameter in order to determine the "vital air" or oxygen in the respired air. The tube was first filled with mercury and inverted over mercury, a little of the gas to be analyzed was introduced and then a bit of phosphorus, which phosphorus was later ignited by bringing a burning ember in the vicinity of the glass. The rest of the air to be analyzed was gradually admitted and when the tube cooled the volume of the air remaining could be measured. The loss in volume represented the quantity of oxygen absorbed. Carbonic acid could then be absorbed by potash. Seguin stated that the older method, as originally introduced by Priestley, had twenty sources of error but that his method merited attention on account of the very great exactitude with which he could determine the gases which are contained in respired air.

He furthermore truly stated that "if we enter into a room containing a large number of people we immediately smell a strong, suffocating odor, but if we use eudiometers to analyze this foul air and compare it with ordinary atmospheric air we find hardly any difference in the proportions of gases which are contained in them."

After Lavoisier's death Madame Lavoisier drew from memory the apparatus used by her husband. The drawings were retouched by David, Madame Lavoisier's instructor in art. There are two pictures quite dissimilar. Good reproductions are to be found in Grimaux's "Lavoisier." In both pictures Seguin sits naked in a chair, breathing through a mask into a series of globes or bell jars. In both pictures Madame Lavoisier is shown seated at a table, taking notes of the experiment. In both pictures the pulse is being counted. In one experiment a weight is placed on Seguin's instep. The arrangement of the apparatus is quite different in the two pictures. In the experiment showing Seguin at work it seems as

though valves were indicated through which inspired air was received from the atmosphere while the expired air was driven through a tube into a bell jar under water. Nysten (Nysten, (*o*) 1817), working in Paris in 1811, described the method by which he caused tuberculous and other patients to respire through valves into a previously collapsed bag for half a minute and then analyzed the expired air by a method similar to that of Seguin.

These are the known historical facts about the apparatus used in the first respiration experiments on man, but the exact details of the method by which results were established and which still are the basis of metabolism studies are unknown.

In contemplating his results Lavoisier (*f*) said: "This kind of observation suggests a comparison of forces concerning which no other report exists. One can learn, for example, how many pounds of weight lifting correspond to the effort of one who reads aloud or of a musician who plays a musical instrument. One might even value in mechanistic terms the work of a philosopher who thinks, the man of letters who writes, the musician who composes. These factors, which have been considered purely moral, have something of the physical and material which this report allows us to compare with the activities of a man who labors with his hands. It is not without justice that the French language has united under the common expression *work* the effort of the mind with that of the body, the work at the desk with the work at the shop. . . .

Thus far we have considered respiration only as a consumption of air, the same kind for the rich as for the poor, for air belongs equally to all and costs nothing. The laborer who works enjoys indeed in great measure this gift of nature. But now that experiment has taught us that respiration is a true process of combustion which every instant consumes a portion of an individual, that this combustion is greater when the circulation and respiration are accelerated and is augmented in proportion to the activity of the individual life, a host of moral considerations suggest themselves from these determinations of physical science.

What fatality ordains that a poor man, who works with his arms and who is forced to employ for his subsistence all the power given him by nature, consumes more of himself than does an idler, while the latter has less need of repair? Why the shocking contrast of a rich man enjoying in abundance that which is not physically necessary for him and which is apparently destined for the laboring man? Let us take care, however, not to calumniate nature and accuse her of faults undoubtedly a part of our social institutions and perhaps inseparable from them. Let us be content to bless the philosophy and humanity which unite to promote wise institutions which tend to bring about equality of fortune, to increase the price of labor, to assure to it just recompense, to offer to all classes of society and especially to the poor more pleasures and greater happiness. Let us trust, however, that the enthusiasm and exaggeration which so readily seize men united in large assemblies, that the human passions which sway the multitude, often against their own interest, and sweep the sage and the philosopher like other men into their whirlpool, do not reverse an outlook with such beautiful vistas and do not destroy the hope of the country. . . .

We end this memoir with a consoling reflection. To merit well of humanity and to pay tribute to one's country it is not necessary to take part in brilliant public functions that have to do with the organization and regeneration of empires. The naturalist may also perform patriotic functions in the silence of his laboratory and at his desk; he can hope through his labors to diminish the mass of ills which afflict the human race or to increase its happiness and pleasure; and should he by some new methods which he has opened up prolong the average life of men by years or even by days he can also aspire to the glorious title of benefactor of humanity.

These are words written by the greatest scientist of his day under the spell of the French Revolution. They are words of an educated, cultivated man of middle age spoken in the Académie des Sciences in the year of the fall of the Bastile and at a time when Edmund Burke from the other side of the Channel said, "In the groves of their Academy at the end of every vista you see nothing but the gallows."

Lavoisier and Franklin had been intimate friends, living near each other in Paris and Franklin dining frequently with the great French chemist and his wife. In a letter written to Franklin, then in America, on February 5, 1790, during the early days of the French Revolution, Lavoisier says: "After having recited what has transpired in chemistry it is well to speak of our political revolution. We regard it as accomplished, well accomplished and beyond recall. There still exists, however, an aristocratic party which is making vain efforts but is evidently feeble. . . . We greatly regret at this moment your absence from France. You could be our guide and mark the limits beyond which we ought not to pass."

And in 1790 Lavoisier (*g*) concluded his last scientific communication to the Académie with these words, "Up to the present time we have learned only to conjecture as to the cause of a great number of diseases and as to the means of their cure. Before hazarding a theory we propose to multiply our observations, to investigate the phenomena of digestion and to analyze the blood both in health and in disease. We will draw upon medical records and the light and experience of learned physicians who are our contemporaries and it will be only when we are thus completely armed that we will dare to attack a revered and antique colossus of prejudice and of error."

No person of understanding can escape a thrill at this vision of modern medicine expressed by him who had overthrown phlogiston, discovered the composition of the air and its relation to combustion and to life, who had created calorimetry and revolutionized the whole of chemical thought.

True to his enthusiasm we find him drawing up the conditions for an international prize of 5,000 livres offered by the Académie des Sciences in 1792 to the author of the best experimental treatise on the liver and the bile (*i*).

Lavoisier's life outside his laboratory had been that of a public

official, a tax gatherer, and he had also been associated with the national manufacture of gunpowder, the quality of which he had greatly improved. He purchased a large landed estate and made experiments in scientific agriculture, doubling the wheat crop, quintupling the number of beasts on the land and earning thereby the enduring gratitude of the peasants. However, as before remarked, he had incurred the bitter hatred of Marat and he was a tax gatherer. In November, 1793, he was arrested at the Arsenal in his laboratory there, upon which he had spent a large portion of his fortune. Just a little while before, in August of the same year, the Académie des Sciences had been closed as inimical to the welfare of the state. *Les amis du peuple* are notoriously suspicious of the "intelligenzia," and the Académie was abolished.

Just prior to his execution Lavoisier wrote to a friend, "I have had a sufficiently long career, always a very happy one, and I believe that my memory will be thought of with some regret and perhaps as having something of glory. What more could I desire? The circumstances which surround me would probably lead to an uncomfortable old age. . . . It is certainly true that all the social virtues, important services to the country, a useful career employed in promoting art and human knowledge, have not sufficed to save me from a sinister end or to prevent me from perishing as a criminal."

One of the charges against Lavoisier was that he had allowed the collection of taxes upon the water contained in tobacco. On May 8, 1794, at the age of fifty years, he was tried and found guilty. Twenty-eight *fermiers-généraux* were executed in the Place de la République at the same time. He witnessed the execution of his father-in-law, Paulze, who was fourth on the list, and he was the fifth upon whom the ax of the guillotine fell.

Such was the Terror.

His friend Lagrange whispered that night to an intimate, "It took but an instant to cut off his head; a hundred years will not suffice to produce one like it!"

Writing a hundred years later, Berthelot (*j*) (1890) exclaimed, "It is our right to admire the positive work which he accomplished. The universal judgment of the civilized world increasingly reveres his establishment of chemistry, one of the fundamental sciences, upon a fixed and definite basis. There is no grander accomplishment in the history of civilization and hence the name of Lavoisier will live forever in the memory of humanity."

It is interesting to consider the differences in the lives of the men concerned in the great discoveries of the last quarter of the eighteenth century. Priestley, an indigent clergyman; Cavendish, of whom it was said that he was the most wealthy of learned men and the most learned of the wealthy; Scheele, a poor Swedish apothecary; and Lavoisier, a man of

affairs, a noble of high social position, in receipt of huge personal revenues. What is it, then, that makes for greatness in science? Would Lavoisier have accomplished more had he been on a "full-time" basis with a restricted income? It is a question of individual opinion, but to most people it would appear that scientific greatness depends primarily upon the quality of the intellectual protoplasm of the brain, upon the advantages offered to the functioning of that brain by a favoring mental environment, and on the possession of a good conscience.

One may well understand that the clarification of the work of Black, Rutherford, Cavendish, Priestley and Scheele by the brilliant mind of Lavoisier might lead others than they to the expression of national scientific self-consciousness. Thus, Wurtz's "Histoire des doctrines chimiques," published in Paris in 1861, begins with the proud statement, "La chimie est une science française; elle fût constituée par Lavoisier." It is needless to state that this caused reverberations of disapproval from England. The personal opinion of national worth finds still more intense modern expression in the Manifesto of the Intellectuals (1915), "The German Mind is, in our opinion, beyond all doubt our one supremely valuable asset. It is the one priceless possession amongst all our possessions. It alone justifies our people's existence and their impulse to maintain and assert themselves in the world; and to it they owe their superiority over all other peoples."

A historic case in which a generous attitude was taken occurred when the French Academy in 1806, just prior to a declaration of war between France and England, conferred its newly established Volta medal upon Humphrey Davy. A French delegation went to London to deliver the medal while the war was in progress and Davy, in acknowledging it, said, "Science knows no country. If the two countries or governments are at war, the men of science are not. That would, indeed, be a civil war of the worst description. We should rather through the instrumentality of men of science soften the asperities of national hostility."

Perhaps this "old-fashioned" courtesy was a relic of the days of a bygone chivalry. At any rate, it affords a delightful example of human behavior.

Science after the French Revolution

Napoleon, during the winter of 1797-1798, attended the regular course of chemical lectures delivered by Berthollet, who had been an associate of Lavoisier. At a later date Berthollet and Monge, the mathematician, organized a company of one hundred scientists to accompany Napoleon to Egypt. At least the scientific men of France had no cause to complain of lack of recognition. And perhaps partly in consequence of this one finds living in Paris in 1823, the year Liebig studied there, such men as La

Place, Berthollet, Gay-Lussac, Thénard, Cuvier, Ampère, Laennec and Magendie.

Thorpe writes of them (1908):

"That constellation has set—

'The world in vain
Will hope to look upon their like again.'"

The atmosphere for the development of French science reached at this time a maximum of power to stimulate. One of the few mistakes of Lavoisier was his conception that oxidation took place in the lungs. Lagrange, the illustrious mathematician, a friend and associate of Lavoisier, reflecting that if the heat production took place in the lungs their temperature must be higher than elsewhere in the body, concluded that heat was generated wherever the blood circulated, that oxygen dissolved in the blood, combined with hydrogen and carbon there, and that carbonic acid was eliminated. This interpretation of Lagrange was published in 1791 before Lavoisier's death by Lavoisier's pupil Hassenfranz (*l*), who agrees that the caloric necessary to maintain animal heat is liberated in the blood by the combination of carbon and hydrogen with oxygen, with which the blood is mixed.

Humphrey Davy (1778-1829) was the first to obtain oxygen from arterial blood by warming it to 93° C. and carbonic acid from the venous blood by warming it to 45° C. He was apparently not well acquainted with Lavoisier's work, and his own work, published in 1799, remained long forgotten. To him oxygen occurred as "phosoxygen," a combination of heat and light. In his experiment XVII he shows that "phosoxygen" can be absorbed by venous blood in the dark without the liberation of light, but with the result that the color of the blood changes from dark red to bright vermilion.

Experiment XVIII.—

A phial containing about 12 inches, having a pneumatic apparatus affixed to it, was filled with arterial blood from the carotid artery of a calf. The phial was placed in a sand bath at a temperature of 96° and the heat gradually and slowly raised. In about ten minutes the temperature of the bath was 108° and the blood began to coagulate. At this moment some globules of gas were perceived passing through the tube. Gas continued to pass in very small quantities for about half an hour when the temperature of the sand was about 200°; the blood had coagulated perfectly and was now almost black. About 1.8 cu. in. of gas were collected in the mercurial apparatus; of this 1.1 cu. in. were carbonic acid and the remaining 0.7 phosoxygen.

From this experiment it is evident that the arterial blood contains phosoxygen, and we have proved before by synthesis that it is capable of combining with it directly. We are possessed of a number of experiments which prove that phosoxygen is consumed in respiration. It has been likewise proved that gases can penetrate through moist membranes like those of which the vessels of the lungs are composed. We may therefore conclude that phosoxygen combines

with the venous blood of the system in the pulmonary vessels. As no light was liberated in Experiment XVII there cannot be even a partial decomposition of phosoxygen in respiration.

Davy's interpretations are far from clear, as will be seen in the following paragraph: "Respiration then is a chemical process, the combination of phosoxygen with the venous blood of the lungs and liberation of carbonic acid and aqueous gas from it. From the combination and decomposition arises an increase of repulsive motion which, combined with that produced by the other chemical processes taking place in the system and that generated by the reciprocal action of the solids and fluids, is the cause of animal heat; a heat which the other systems have supposed to arise chiefly from the decomposition of phosoxygen (oxygen and caloric)."

About the same time that Davy was experimenting in England Spallanzani in Italy was inquiring into the validity of Lavoisier's ideas.

Spallanzani (1729-1799).—The experiments of Spallanzani were published in 1804 after his death. His biographer states: "When the Empress Maria Theresa had reëstablished the University of Pavia on a more extensive plan she wished to render it at once celebrated by the attainments of its professors; she empowered Count Firmian to invite Spallanzani to give lectures on natural history."

Spallanzani says that oxygen is transported by the blood to the heart and is necessary for the heart beat, but he is not convinced that oxygen is necessary for the production of carbonic acid. He put snails into two tubes filled, respectively, with atmospheric air and with nitrogen. "On removing them from the tubes at the end of twelve hours I found the animals still alive; I examined the two aëriform fluids and was astonished to discover that the quantity of carbonic acid gas was greater in the azotic gas (nitrogen) than in the common air." He obtained the same result when he used hydrogen gas and says, "I shall only conclude from these experiments that it is clearly proved that the carbonic acid gas produced by the living and dead snails in common air resulted not from atmospheric oxygen, since an equal or even a greater quantity of it was obtained in azotic and hydrogen gas."

This is very nearly the same as Davy's conclusion. Of his method of work Spallanzani says: "Being engaged in similar experiments, it was natural for me to attend to this part of the subject uninfluenced by the opinion of those celebrated men, in order that I might observe only nature herself. This is at least the mode I have always pursued, when it was possible, with respect to the most universally received opinions, however respectable the quarter whence they proceeded; I have always myself examined the facts on which they were built."

William F. Edwards (1776-1842) confirmed the work of Spallanzani, finding that frogs when placed in hydrogen gas eliminated in a few hours a

volume of carbonic acid equal to their own volume and larger in quantity than they would have expired had they breathed in air. He concluded that carbon dioxid was not formed by oxidation in the lungs but must have been excreted from the blood, and he supports this conclusion by citing unpublished experiments by Vauquelin in which blood was exposed to a hydrogen atmosphere with the result that carbonic acid was given off.

Magnus (1802-1870) repeated the experiments of Vauquelin, shaking blood in hydrogen gas, and he also placed blood in a complete vacuum and noticed the elimination of a great volume of gases. There was more carbonic acid eliminated than could be accounted for by the bicarbonate present.

Gay-Lussac (1778-1850) criticized these results and stated that the quantity of oxygen found in the blood was sixteen times larger than could be dissolved by water and that no differences appeared in the analyses of arterial and venous bloods. Magnus (1845) replied that 100 parts of gas extracted from blood contained:

	Arterial Blood	Venous Blood
Carbonic acid	62.3	71.6
Oxygen	23.2	15.3
Nitrogen	14.5	13.1
	100	100

He found also that when blood was pumped out it could again absorb sixteen volumes per cent of oxygen.

Berzelius (1779-1848) announced in 1838 that little oxygen could be added to blood serum freed from corpuscles, but when the serum was mixed with the coloring matter of the blood it was absorbed in large volume. Berzelius attributed the affinity of "hematin" for oxygen to its content of iron.

Dumas in 1846 found that on replacing blood serum with a solution of sodium sulphate the blood corpuscles suspended therein still changed in color after shaking with oxygen.

It was Liebig in 1851 who gave expression to modern thought upon the subject of the respiration in saying, "The absorption of a gas by a liquid is due to two causes, an external consisting in the pressure exerted by the gas upon the liquid, and a chemical, an attraction manifested by the constituent particles of the liquid."

For complete references to this story, consult "Lecons sur la physiologie," by H. Milne-Edwards, Volume 1, printed in 1857. These volumes treat the subject of physiology with a thoroughness lately thought to be exclusively German.

The Beginnings of Calorimetry

The work of Lavoisier concerning the source of animal heat was insufficiently convincing, and so the French Academy of Science offered a prize to any one who would produce the best thesis on the subject. The prize was competed for by Despretz and by Dulong. It was awarded in 1823 to the former, although in the light of modern knowledge it would seem that the latter had a greater insight into the problem.

Despretz (1792-1863) gives the following account (1824): "No phenomenon in physiology is more capable of attracting attention than the singular property enjoyed by man and warm-blooded animals of preserving an almost constant temperature, although the temperature with which they are surrounded is subject to continual variations. All bodies tend constantly to seek heat equilibrium; reciprocal exchange tends to establish a uniform temperature between different bodies.

"Warm-blooded animals, on the contrary, though they are equally exposed to heat loss occasioned by contact, radiation and the evaporation of water, possess within themselves a power to produce heat which maintains their temperature as a rule at about 39° above the melting point of ice."

The resources of modern science were lacking in the days of Galen, Boerhaave and Haller. The author cites Lavoisier (*n*) (1780) and criticizes Crawford's (1779) very imperfect method. He states that Brodie (1812 Philosophical Transactions) thought the brain produced heat through the nerves, citing the heat loss after decapitation. This was denied by Le Gallois, who maintained artificial respiration in a decapitated animal.

Type of experiment by Despretz:

Subjects, three guinea-pigs.
Ventilation, 55 to 60 liters per 2 hours, the air being purified by passing through potash.
Condition of the environmental air, 6 per cent CO_2 and water saturation.

Experiment 1:

CO_2 formed, 2,587 liters.
O_2 unaccounted (i. e., not in CO_2), 0.709 liter.
The three animals raised the temperature of 23310.5 g. water 0.63°.

Animal heat as measured,	100 per cent.
Heat due to formation CO_2	69.9 per cent.
Heat due to formation water,	19.4 per cent
Total heat as calculated,	89.3 per cent.

The modern calculation would be:

O_2	CO_2	R. Q.	Calories indirect	Calories direct
liters	liters			
3.30	2.59	0.78	15.86	14.68

Or 8 per cent too much calculated heat instead of 11 per cent too little.

The conclusions of Despretz were:

1. That the respiration is the principal cause of the development of animal heat; that assimilation, movement of the blood, friction in different parts, can easily produce the small residual amount.

2. Although oxygen is employed in forming carbonic acid, a certain quantity, sometimes considerable in amount, disappears; it is generally thought that it is used in the combustion of hydrogen.

3. There is an exhalation of nitrogen in the respiration of both carnivorous and herbivorous animals.

The following animals were used: Ducks, chickens, cocks, young and old pigeons, gulls, buzzards, owls, magpies, dogs, cats, rabbits and guinea-pigs.

Dulong (1785-1838) presented the second paper in competition for the prize of the Academy, of which a résumé follows:

The author, who is both physicist and chemist, proposes to determine if the quantity of oxygen intake is sufficient (in health) to repair the heat loss by animals under natural conditions of life; in other words, whether animal heat is entirely due to combustion which takes place within the animal through respiration.

He calls attention to the fact that Lavoisier used two different guinea-pigs, one in the calorimeter and another for the determination of the gaseous exchange. He uses the water calorimeter of Rumford. The temperature of the water is the same as that of surrounding air at the start; at the end, higher. The animals can move at will. Cat, dog, kestrel, capibara (water-hog), rabbit, and pigeon are used. He finds that in the cat, dog and kestrel the volume of oxygen inspired is one-third more than that of the carbonic acid expired, whereas in rabbits, capibara and pigeons the oxygen is only one-tenth more than the carbonic acid. Therefore he thinks this difference is due to food or to a difference of animal organization through food. He finds that nitrogen is exhaled. The heat from carbonic acid in carnivora is 49 to 55 per cent of the total heat measured; in herbivora, 65 to 75 per cent. Calculated inclusive of the heat produced from the oxidation of hydrogen, it equaled 69 to 80 per cent. The experiments were repeated many times.

One source of error in the calculations of Despretz and of Dulong

lay in the fact that the caloric values attributed to the oxidation of carbon and hydrogen were wrong. One may compare the values used at different periods as follows:

	Lavoisier 1780	Despretz 1823	Favre and Silbermann 1852-53
	calories	*calories*	*calories*
1 gm. H oxidized yields...	22.170	23.640	34.462
1 gm. C oxidized yields...	7.237	7.914	8.080

The agreement between Despretz and Dulong that nitrogen was present in the expired air in an amount larger than that inspired was accepted for many years by many writers. Magendie, in his "Elements of Physiology," in 1836, thus expresses the thoughts of his time: "According to the experiments of M. Despretz upon herbivora, the respiration furnishes only 89 per cent of the animal heat, and in carnivora only 80 per cent. Therefore, other sources of animal heat must exist in the economy. It is probable that these occur in the friction of various parts, in the movement of the blood, the friction of the blood corpuscles upon one another and finally in nutritive phenomena. This supposition is not forced, for it is known that most chemical combinations give rise to heat, and it is doubtless true that combinations of this nature take place in the organs, both during secretion and digestion."

It is evident that ignorance of the Law of the Conservation of Energy hampered progress at this time.

Dumas (1800-1884).—In the year 1823 a paper was published by Prévost and Dumas pointing out the fact that if the kidneys were extirpated in cats and rabbits, urea rose to high concentration in the blood. This experiment proved that urea was not formed in the kidney. Rouelle in 1773 had found urea in the urine.

It was the year 1823, the year of the publication of the work of Despretz, of Dulong and of Dumas, that Liebig, at the age of twenty, came to Paris to study. This should be remembered as the story of the development of the French school is unfolded. The part Liebig played will be told later.

Dumas was an organic chemist of high repute. Concerning his influence, the words of Pasteur, spoken in 1882, may be recalled: "My dear Master, it is indeed forty years since I first had the happiness of knowing you and since you first taught me to love science.

"I was fresh from the country; after each of your classes I would leave the Sorbonne transported, often moved to tears. From that moment your talent as a professor, your immortal labors and your noble character have inspired me with an admiration which has grown with the maturity of my mind."

Dumas came into frequent intellectual conflict with Liebig and Wöhler in Germany and Berzelius in Sweden. In 1828 Wöhler produced urea synthetically from ammonium cyanate, delivering the final death blow to the doctrine that organic compounds arise only through the intervention of living things.

Magendie (1783-1855) was among the first to differentiate between various kinds of foods. This distinguished physiologist fed dogs cane sugar or olive oil or butter and found that death occurred in 34 days (Magendie, 1836). He rightly concluded that the nitrogen of the organs of the body arose only from the nitrogen of the food, that the nitrogen-free food-stuffs were not transformable into nitrogen-containing food-stuffs. He rendered great service in pointing out the nitrogen content of rice, maize and potatoes, foods upon which people live.

Magendie also found that dogs fed with bread alone lived only a month. The second gelatin commission of the French Academy (Magendie, 1841), sitting in 1841 under the presidency of Magendie, determined that bread and gelatin given together to either dog or man constituted an insufficient diet.

Boussingault (1802-1887).—Organic analysis, which was founded by Lavoisier, was further advanced by Gay-Lussac and Thénard (1810-15), by Berzelius in 1814, and was perfected by Liebig in 1830. This work led to that of Boussingault, who curiously enough had been previously for several years in the employ of an English mining company in equatorial South America.

The experiments of Boussingault in 1839 may be considered to be prophetic of the future evolution of metabolism studies. Boussingault compares the quantities of carbon, hydrogen, nitrogen and oxygen in the fodder constituting a maintenance ration of a milch cow, with the quantities of the same elements eliminated in the urine, feces and milk. The difference between these quantities would be available for the respiration. He gives the following account (Boussingault, (*b*) 1839):

"It is generally recognized to-day that the food of animals must contain a certain amount of nitrogen. The presence of nitrogen in a large number of vegetable foods forces the conclusion that herbivora receive nitrogen in their food, which enters into their constitution.

"In ordinary alimentation an individual does not change his average weight; this state of affairs exists when an animal takes a *maintenance ration* (*ration d'entretien*)."

Under these conditions the food of the animal should be found in his excretions. During growth, or the process of fattening the conditions would be different.

Cows were given a maintenance ration of known elementary composition and the elements recovered in the urine, feces and milk were subtracted from those in the fodder, with the following results:

	C	H	O	N	Salts
Elements in the fodder.....	4813	595	4035	201.5	889
Elements in the urine, feces and milk.	2602	332	2083	174.5	921
	—2211	—263	—1952	—27	+32

Uniting the oxygen of the food with the hydrogen in such a proportion as to form water, there would remain 19.8 gm. of hydrogen requiring inspired atmospheric oxygen for its conversion into water. The loss of carbon equaling 2211 gm., it would require 4052 liters to convert it into 7999 gm. of carbonic acid. A cow would therefore deprive 19 square meters of air of its oxygen.

Boussingault states that one nitrogen determination is not sufficient to decide whether nitrogen as a gas enters into the metabolism of protein.

The same kind of work is done with a horse (Boussingault, (*a*) 1839). It is concluded that 4584 liters of oxygen would be required to form the carbonic acid produced. There were 24 gm. less of nitrogen in the excreta than in the food. It seems clear that atmospheric nitrogen is not assimilable by the body.

In a subsequent experiment published in 1843 Boussingault (*c*) gives food to a turtle-dove and estimates the carbonic acid elimination as he had done with the horse, but he also determines directly the carbonic acid given off. By the first method 0.211 gm. of carbon were estimated to have been expired and by the second method an average of 0.198 gm. were actually found. This closely approaches modern technic.

Boussingault and Le Bel (1839) made the first complete analyses of cow's milk. They conclude from their work that the nature of the fodder does not affect the quantity or the chemical composition of the milk, provided the cow receives the same relative nutritive equivalents in the fodder.

The nutritive equivalents, however, were based on the nitrogen content of the fodders, thus 13.5 kg. of hay were accounted the nutritive equivalents of 54 kg. of beets or 27 kg. of potatoes. It is evident that at this date there was no real understanding of the nature of the different foodstuffs.

Barral (1819-1884) in 1849 applied the principles of Boussingault's method to the analysis of the metabolism of human beings. He thus presents his problem: "Knowing the amount and the elementary composition of the food, both solid and liquid, taken each day, determining the elementary composition of the excreta and perspiration, one may calculate the gains and losses of the human body."

His experiment on himself lasted five days, with the following results per day:

	Water	Salts	Cl	C	H	N	O	Total
In the food.....	1998.6	31.3	7.8	366.2	57.3	28.0	265.7	2754.9
In the excreta....	1177.8	15.4	5.0	30.5	5.4	13.7	16.9	1264.7
Differences	—820.8	—15.9	—2.8	—335.7	—51.9	—14.3	—248.8	—1490.2

248.8 g. O_2 + 31.1 g. H_2 = 279.9 g. H_2O

20.8 g. H_2 + 166.3 g. insp. O_2 = 187.1 g. H_2O

335.7 g. C + 895.2 g. insp. O_2 = 1230.9 g. CO_2

It is evident that 1061 gm. of oxygen would have been inspired and 1231 gm. of carbonic acid expired, according to this calculation. He finds that his figures for carbonic acid elimination accord with those of Andral and Gavarret (see below). He calculates the heat production as follows:

335.7 g. C × 7.200 calories = 2417.040 calories from C
20.8 g. H × 34.600 " = 719.680 " " H

Total 3136.720

These calories were calculated for a man from the food partaken during the winter months.

Barral makes the further analysis of the heat produced by various individuals in 24 hours:

Subject.	Total calories	Calories per kgm.
Barral, in winter (age 29 yrs.; wgt. 47.5 kgm.)...	3,136.720	66.036
Barral, in summer.....	2,312.000	48.673
Barral's son (age 6 yrs.; wgt. 15 kgm.).....	1,223.960	81.597
Laboratory servant (age 59 yrs.; wgt. 58.7 kgm.).	2,559.080	43.595
Woman (age 32; wgt. 61.2 kgm.).....	2,541.100	41.521

The quantity of nitrogen in the food was always greater than that found in the evacuation, so much so that a part must have been eliminated in the respiration. This portion was one-third or one-quarter of the nitrogen taken in the food but was not more than the hundredth part of the volume of carbonic acid eliminated. The loss of food nitrogen was estimated as not more than six ten-thousandths of the total volume of air expired.

Barral did not know that his urinary nitrogen analyses were faulty.

Barral criticizes the contemporary work of Liebig as follows: "Liebig has attempted the solution of the question which occupies us by the same method and as concerns man. This skilful chemist was content to measure the *principal* foods of a company of the grand ducal guard of Hesse-Darmstadt and to regard the minor food-stuffs as the approximate equivalent of the material found in the feces and urine so far as carbon content was concerned. He also made similar valuations of the food-stuffs of prisoners at Giessen and at Marienbad and of a family composed of five

persons. But this application of the method of Boussingault is too imperfect to establish definitely incontrovertible results in science."

It might be added at this point that Liebig in 1845 found that nine-tenths and more of the heat measured by the calorimeters of Dulong and of Despretz could be accounted for from the oxidation of carbon and hydrogen calculated according to the method of Lavoisier. The more modern caloric values for hydrogen were here employed as later in 1855 by Gavarret.

Liebig also points out that if one of the dogs experimented upon by Dulong had really eliminated the quantity of nitrogen gas Dulong had reported, the animal in seven days would have expired as nitrogen gas the amount of that element contained in its hair, skin, flesh and blood, and at the end of the period would have been merely a mass of mineral ash.

Regnault (1810-1878).—Henri Victor Regnault was born in Aix-la-chapelle, and in 1840 became professor of physics and chemistry at the University of Paris. In 1847 he became also chief engineer of mines; in 1854 was director of the Sèvres porcelain manufactory. He was a strict disciplinarian of students and up to the outbreak of the war in 1914 his memory was held in tradition as representative of the highest pedagogical severity.

In 1849 *Regnault and Reiset* published their celebrated monograph upon the respiration of animals. The apparatus which they used consisted of a closed system, from which the carbonic acid produced by an animal placed within the system could be absorbed, and into which oxygen could be admitted as the atmospheric air was consumed by the animal. This is the "closed system of Regnault and Reiset," the principle of which is employed in modern calorimeter work (*vide* Atwater and Benedict, 1905).

The results obtained were usually accurate and their interpretations were within the compass of the knowledge of the time.

Their main conclusions as they enumerated them, together with some of their experimental data, are presented in the following abstract:

For animals of warm blood, mammals and birds:

1. Normally nourished animals constantly expire nitrogen but the quantity eliminated is very small, never exceeding two per cent and often being less than one per cent of the total oxygen consumption.

2. If animals fast they frequently absorb nitrogen. The proportion of nitrogen absorbed varies within the same limits as the exhalation of nitrogen by animals regularly fed. This absorption of nitrogen takes place in almost every instance in the case of birds but scarcely ever in mammals. . . .

(In experiment 10 performed on a rabbit the quantity of nitrogen absorbed was 0.08 per cent of the quantity of oxygen absorbed. In the text of the article they remark that the enormous elimination of nitrogen

reported by Dulong is impossible and that Liebig had pointed out [p. 40] that when one considered the loss of nitrogen in the urine and feces, an animal expiring in addition the amount of nitrogen found by Dulong would thus in a few days liberate all the nitrogen contained in the organic material of its own body. They also state that the respiration cannot contain more than extremely small quantities of ammonia.)

4. . . . The alternating elimination and absorption of nitrogen found in the same animal under various conditions is favorable to the opinions

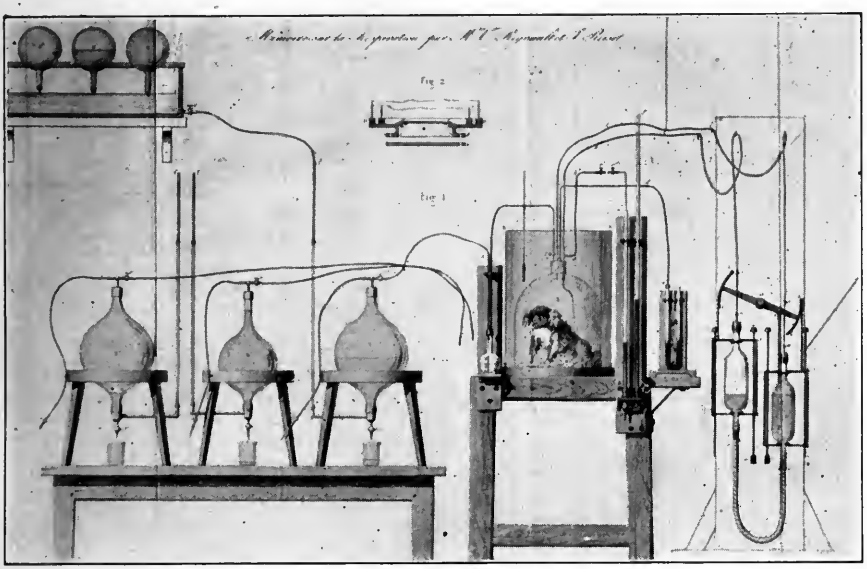


Fig. 6. The closed circuit apparatus of Regnault and Reiset. From "Annales de Chimie et de Physique," Series 3, Vol. XXVI, Pl. III. Water rising in the glass receptacle drives oxygen into the glass bell jar. A pump alternately raises and lowers two cylinders. The lower cylinder fills with alkali at the expense of the upper one, and this movement of the liquid forces air from one cylinder into the bell jar and draws a corresponding amount from the bell jar into the other cylinder.

of Edwards, who believes that an elimination and an absorption of nitrogen constantly takes place during respiration, and what one finds is the resultant of these two contrary processes.

5. The relation between the quantity of oxygen exhaled as carbon dioxid and the quantity of total oxygen consumed appears to depend more on the nature of the food than on the species of the animal. This ratio is higher in the animals which live upon grain and in them it may exceed unity. When they are given meat, the ratio is less and varies between 0.62 and 0.80. Upon a diet of legumes the ratio is between that found after giving meat and that after giving bread.

6. This ratio is nearly constant in animals of the same race, such as dogs when they are given the same diet.

7. Fasting animals show about the same ratio (R. Q.) as they do when fed with meat, though usually a little less than under latter conditions. During inanition fasting animals live off their own flesh, which is of the same nature as the flesh which they eat. All fasting animals present the picture of carnivora.

8. The fact that the relation between the volumes of oxygen absorbed and carbonic acid exhaled varies between 0.62 and 1.04 according to the kind of food which the animal takes in, destroys the validity of the hypothesis of Brunner and Valentin (1846), attributing the respiration to the simple diffusion of gases through membranes according to the laws of Graham (which calls for a constant ratio of 0.85). In the text they describe how they placed the bodies of animals (fowls, dogs, rabbits) in an impermeable rubber sack and found in mammals, as well as in birds, that the total quantity of carbon dioxid eliminated from the skin and intestine of these animals was practically negligible, rarely exceeding two per cent of that found in the pulmonary respiration.

9. Lavoisier tried to prove that the heat of the body came from the oxidation of carbon and hydrogen. Regnault and Reiset do not doubt that the heat is in fact derived *entirely* from chemical reactions in the body. But they think the reactions are too complex to be computed on the basis of the oxygen intake. "The substances which are oxidized are composed of carbon, nitrogen, hydrogen, and often contain a considerable amount of oxygen. Though they be completely oxidized in the respiration process, their own oxygen content contributes to the production of water and carbonic acid, and the heat which is liberated is necessarily different from that which would have been evolved by the oxidation of carbon and hydrogen supposedly liberated. Moreover, the food substances are not completely destroyed, for portions are converted into other materials which play a special part in the body's economy and portions are transformed into urea and uric acid. In all the transformation and assimilative processes which these substances undergo in the organism there is either liberation or absorption of heat; but the processes are evidently so complex that it is very unlikely that one will ever be able to calculate them."

(They found in fowls that the volume of carbon dioxid was often greater than the volume of oxygen, which rendered the proposition of estimating the heat production from the oxygen impossible.)

10. The quantity of oxygen varies during different periods of digestion because of muscle work, and numerous other circumstances. In animals of the same species and the same weight the quantity of oxygen is larger in young individuals than in adults. It is greater in healthy, thin animals than in fat ones.

11. The consumption of oxygen absorbed varies greatly in different animals per unit of body weight. It is ten times greater in sparrows than in chickens. Since the different species have the same body temperature

and the smaller animals present a relatively larger area to the environmental air, they experience a substantial cooling effect, and it becomes necessary that the sources of heat production operate more energetically and that the respiration increases.

14. Awakening marmots consume oxygen in very largely increased quantity.

17. Reptiles consume much less oxygen per unit of body weight than do warm-blooded animals, but do not differ from them in the relative quantities of oxygen and carbon dioxid.

18. Frogs without lungs respire just as well as frogs with lungs.

19. Frogs and earthworms show nearly the same metabolism per kilogram of body substances.

20. The respiration of insects, such as beetles and silkworms, is very much more active than that of reptiles. For equal body weights they consume as much oxygen as mammals, and a proportionately large amount of nourishment. We are comparing insects with animals two to ten thousand times heavier than they.

A thermometer placed in the midst of a mass of active beetles inclosed in a sack showed a temperature of two degrees higher than the surrounding air.

The results of the work on these lower forms of life may be thus summarized:

	Weight gm.	R. Q.	Oxygen per kg. per hr.	Temp.
37 Beetles	37.	0.82	0.962	
18 Silkworms ..	42.5	0.79	0.840	
25 Chrysalides..	21.	0.64	0.240	
— Earthworms.	112.	0.78	0.101	
2 Frogs	127.5	0.75	0.105	19°

21. Animals of different species respire just the same in air containing two to three times the usual quantity of oxygen, and do not perceive the difference in oxygen content. (The air contained 72.6 per cent of oxygen.)

22. If hydrogen replaces nitrogen of atmospheric air there is very little difference in the respiration process. (The air contained 77 per cent of hydrogen and 21.9 per cent of oxygen.)

There were 104 experiments in all.

Regnault and Reiset exemplify their natural instincts of friendship and courtesy when they write that experiment 26, in which they varnished a dog with gelatin, was done at the suggestion of "cet habile physiologiste Magendie," and that M. Bernard "dont l'habilité est bien connue de tous les physiologistes" had extirpated the lungs of the frogs about half an hour before placing them in their apparatus.

In the closing words of this masterpiece the authors write:

> We are far from concluding that our work presents a complete study of respiration. We consider ourselves happy if we have established the principal facts and if our methods are useful to physiologists who, through their special learning, may be able to extend them.

The animals were never inconvenienced in any way in the apparatus. Though single animals were often used in many experiments, there was never any deleterious effect upon their health.

> It will be noticed that there are two regrettable omissions in our work, experiments on the respiration of fish and of man. We have not made experiments on fish because we knew that Valenciennes was doing this. Regarding the respiration of man it was our intention to accomplish this in a special research. We proposed to study not only healthy men under various conditions of diet and at rest or at work, but also patients affected with different diseases and we hoped to associate ourselves in this important work with one of the skilled physicians of the large Paris hospitals. Unfortunately, the new apparatus which was to have served for this investigation, on account of the special conditions it had to satisfy, cost more money than we had at our disposal and we had to renounce our project.
>
> The study of the respiration in man in its various pathological phases appears to us to be one of the most important subjects that could occupy those who follow the art of healing the sick; it can give a precious means of diagnosis in a great number of diseases and render more evident the transformations which take place in the organism. . . . Our desires will be fulfilled if our work provokes study that will be of such great importance to humanity.

The Rise of German Science

Justus von Liebig (1803-1873).—It has already been stated that Liebig was in Paris during the greatest period of French scientific achievement. Liebig had been a dunce at school and was laughed at by his teacher when, as a boy, he expressed his determination to become a chemist. Liebig attended the university of Erlangen, where he was duly educated in the spirit of the phlogiston hypothesis. He heard with impatience the lectures of the renowned philosopher Schelling, and found no satisfaction until, in the autumn of 1822, he went to study in Paris (see p. 36). Both Liebig and Dumas were introduced into the scientific circles of Paris by Alexander von Humboldt. Liebig, dedicating a French edition of one of his books to Thénard, a former master, thus expresses his appreciation:

> "To Monsieur le Baron Thénard,
> Member of the Académie des Sciences.
> Monsieur:
>
> "In 1823 when you presided over the Académie des Sciences a young foreign student came to you and begged you to advise him concerning the fulminates which he was then investigating.

"Attracted to Paris by the immense reputation of those celebrated masters whose glorious researches established the foundations of the sciences and elevated them into an admirable edifice, he had no other introduction to you except his love of study and his fixed desire to profit from your teachings.

"You bestowed on him a most encouraging and flattering welcome, you directed his first researches, and through your influence he had the honor to communicate them to the Académie.

"It was the session of the 28th of July which decided his future and opened a career in which for seventeen years he has labored to justify your benevolent patronage.

"If his labors have been useful, it is to you that science is indebted for them, and he feels obliged to express publicly to you his ineffaceable sentiments of gratitude, esteem and veneration."

JUSTUS LIEBIG.

Giessen, 1 January, 1841.

Through the influence of Alexander von Humboldt, Liebig was appointed professor of chemistry at Giessen in 1824 at the age of twenty-one. Wilhelm Ostwald writes in his "Grosse Männer" that this gave him free water to swim in. Here he built the first modern chemical research laboratory and attracted to it men, many of whom afterward became distinguished. Liebig's "Thierchemie in Ihrer Anwendung auf Physiologie und Pathologie" was first published in 1840 and passed through nine editions. Comparison should be made between it and the publications of Boussingault already described.

Liebig divided the foodstuffs into protein, fat and carbohydrate, and stated that protein could take the place of body protein, while carbohydrate and fat could spare body fat. He believed that muscular work caused the metabolism of protein, while oxygen destroyed fat and carbohydrate.

In the introduction he states that in fifty years it will be as impossible to separate chemistry from physiology as it was then to separate chemistry from physics; that he had endeavored to bring chemistry and physiology together in a single book.

In one of his writings Liebig says that the acceptance of principles, like the application of chemistry to physiology, all depends on the mental development, that the great Leibnitz refused to accept Newton's doctrine of gravitation, which is now understood by every schoolboy.

The time was propitious for the writing of Liebig's book. He himself had been more largely the creator of organic chemistry than any man then living. Chemical compounds of carbon were becoming known. Scheele had discovered uric acid and lactic acid in 1776 and glycerin as a component of fat in 1778; Fourcroy and Vauquelin in 1779 and Prout in 1803 had analyzed urea; Chevreul announced the chemical constitution of fat in 1823 and Thénard investigated the composition of bile; Berzelius, the composition of the secretions in general. In 1828 Wöhler prepared

urea synthetically, and in 1837 Liebig and Wöhler, working together, described the decomposition products of uric acid.

Carl Voit, writing in 1865, thus describes Liebig's services:

All these chemical discoveries, to which Liebig so largely contributed, gave him his fruitful conceptions concerning the processes in the animal body. Before him the observations were like single building-stones without interrelation, and it required a mind like his to bring them into ordered relation. It is a service which the physiologists of our own day do not sufficiently recognize. In order to appreciate this one has only to read physiological papers written before the publication of his books and afterward in order to witness how his writings changed the mental attitude toward the processes in the organism. The chemical discoveries on which he based his conclusions were, in fact, matters of general knowledge, but it was he who applied them to the processes of living things. Scientific progress is determined by the establishment of correct interpretations and the creation thereby of new pathways and problems. A school-boy has a better knowledge of many things than the wisest man had formerly; and he laughs at the ignorance of his forefathers because he does not understand the history of the human mind.

The man of science ought to realize the factors which have given him the vantage which he holds. But there are textbooks on physiology in which the chapters on the animal mechanism do not even mention the name of Liebig. This anomaly is possible only for those who do not understand history, and who hold only the new to be worthy of consideration. Liebig was the first to establish the importance of chemical transformations in the body. He stated that the phenomena of motion and activity which we call life arise from the interaction of oxygen, food and the components of the body. He clearly saw the relation between metabolism and activity and that not only heat but all movement was derived from metabolism. He investigated the chemical processes of life and followed them step by step to their excretion products.

The following quotations from Liebig's (*b*) "Thierchemie" appear to be significant of his attitude (Cambridge, 1842; Braunschweig, 1846):

It is clear that the number of heat units liberated increases or decreases with the quantity of oxygen given to the body in a given time through the respiratory process. Animals which respire rapidly and are therefore able to absorb a great deal of oxygen can eliminate a larger number of heat units than those which have the same volume but absorb less oxygen.

Of metabolism in fasting, he writes:

The first action of hunger is a disappearance of fat. This fat is present neither in the scanty feces nor in the urine, its carbon and hydrogen must have been eliminated through the lungs in the form of oxygen-compounds. It is clear that these constituents are related to the respiration.

Oxygen enters every day and takes away a part of the body of the fasting person with it.

Martell found that a fat pig lived 160 days without food and lost 120 pounds.

In herbivora ten volumes of oxygen absorbed result in nine volumes of carbon dioxid eliminated. In carnivora only six or five volumes carbon dioxid are eliminated (Dulong and Despretz).

With the exception of a small amount of sulphur, hydrogen is the only other combustible substance with which oxygen could combine and it can be regarded as settled that, whereas in the body of an herbivorous animal one-tenth of the oxygen is used to form water, in the body of the carnivorous animal four or five times that quantity are so employed.

In the exact analysis of the process of respiration it is evident that the carbon dioxid production is related to water formation and the two cannot be dissociated. It is therefore self-evident that the determination of the quantity of carbon dioxid expired by an animal within a given time is not a measure of the respiratory process and that all experiments in which the relation of the food to the total oxygen intake is not considered have only a relative value.

In starvation it is not alone fat which disappears but also all solids which are capable of solution. In the completely wasted body of the fasting man the muscles become thin and soft, lose their contractility; all parts of the body which were capable of producing movement have served to protect the rest of the organs of the body from the destroying influence of the atmosphere. Finally the particles of the brain become involved in the oxidation process, delirium, madness and death follow; resistance completely ceases, chemical putrefaction ensues, and all parts of the body unite with the oxygen of the air.

Liebig speaks of the cleavage of sugar into lactic acid, into alcohol and carbonic acid, and later into butyric acid, hydrogen and carbonic acid. He then remarks:

No one will deny that such influences are at work not only in the respiratory process but also have a part in the processes which take place in the animal body, and if further investigations demonstrate that the cause of the decomposition of sugar into alcohol and carbonic acid in alcoholic fermentation is dependent on the development of a lower order of vegetation, and that the metabolism of complex molecules with the production of new substances can be caused by contact with certain particles which are in the state of vital movement, it is clear that a pathway has been constructed which leads to a vision of the mysterious processes of nutrition and secretion.

As to the energy production, he says:

The lack of a correct viewpoint regarding energy and activity and their relation to natural phenomena, has led people to ascribe the production of animal heat to the nervous system. If one excludes the metabolism within the active nerves, the above proposition would be merely saying that movement would arise from nothing. But out of nothing no power or activity can arise.

Liebig asks:

What is the use of fat, butter, milk-sugar, starch, cane-sugar in the diet? Through these non-nitrogenous food-stuffs a certain amount of carbon and in the case of butter a certain amount of carbon and hydrogen are added to the nitrogen-containing materials and form an excess of elementary substances which cannot be used to generate nitrogen- and sulphur-containing substances, which latter are contained preformed in the food. Hardly a doubt can be entertained

that this excess of carbon or of carbon and hydrogen is expended in the production of animal heat and serves to protect the organism from being attacked by atmospheric oxygen.

Further on he remarks:

In their final forms meat and blood which are consumed yield the greater part of their carbon to the respiration, their nitrogen is recovered as urea, and their sulphur as sulphuric acid. Before this occurs the dead meat and blood must be converted into living flesh and blood. The food of carnivora is converted into blood which is destined for the reproduction of organized tissue.

We know that the nitrogen-containing products of metabolism are not susceptible of further change and are eliminated from the blood by the kidney.

Differences in the quantity of urea secreted in these and similar experiments are explained by the condition of the animal in regard to the amount of the natural movement permitted. Every movement increases the amount of organized tissue which undergoes metamorphosis. Thus, after a walk, the secretion of urine in man is invariably increased.

In the animal body the components of fat are used for the respiration process and hence for the production of animal heat.

If the condition and the weight of all parts of a carnivorous animal are to be maintained it must daily receive a certain definite measure of sulphur and nitrogen-containing food substances as well as of fat.

The difficulties of calculating the metabolism are discussed.

The weight of the ingested materials must be the same as those eliminated in the forms of uric acid, urea, carbonic acid and water. The weight of the ingested fat must be the equivalent of the fat eliminated in the form of carbonic acid and water. From this it follows that the quantity of oxygen absorbed cannot be a measure of the amount of the living substance destroyed in a given time.

The oxygen absorption expresses the sum of two factors; one the destruction of nitrogen-free substances and the other the destruction of nitrogen-containing substances. It has already been frequently stated that the measure of the latter can be determined from the nitrogen content of the urine.

He later considers the metabolism of a horse: "A horse preserves itself in a state of health if he be given 7½ kg. hay and 2¼ kg. oats. Hay contains 1.5 per cent and oats 2.2 per cent of nitrogen. Assuming that all the protein in the food is transformed into the fibrin and serum albumin of the blood, there would be produced daily 4 kg. of blood, containing 20 per cent of water and 140 gm. of nitrogen. The quantity of carbon combined with the protein and ingested at the same time would have been 448 gm. Of this only 246 gm. could have served for the respiration, for 95 gm. are eliminated in the form of urea and 109 gm. in the form of hippuric acid. . . . The experiment of Boussingault which shows that a horse expires 2450 gm. of carbon in a day cannot be very far from the truth."

The nitrogen-containing substances of the fodder of the horse do not contain more than one-fifth of the carbon necessary for the maintenance of the

respiration, and we see that the wisdom of the Creator has added to all the foods the remainder of the carbon in the form of sugar, starch, etc., which is necessary for the renewal and maintenance of animal heat and for the conversion of inspired oxygen into carbonic acid. If these substances had not been present in the food and there had been the same intake of oxygen, then the materials of the animal's own body would have been used instead.

Liebig says that only a small fraction of the bile is unabsorbed and cannot contribute greatly to the formation of the feces.

As to the formation of fat, Liebig argues as follows:

A spider, fieree with hunger, sucks the blood of the first fly, but is not to be disturbed by a second or third fly. A cat eats the first and perhaps a second mouse, and will kill but not eat a third. Lions and tigers react the same way, driven by hunger to devour their prey.

How different with a sheep and a cow in the pasture, which eat almost without intermission as long as the sun in the heavens shines upon them.

The herbivorous animals eat in such excess that the ingestion of starch is greater than is necessary for union with oxygen, and hence the animals fatten through conversion of starch into fat.

Concerning alcohol, he makes the following comments: "Alcohol is oxidized in the body, the carbon dioxid elimination decreases after alcohol (Vierordt) because relatively more oxygen unites with hydrogen."

Liebig has been informed that in England all servants are given beer, or where the Temperance Society is influential the money equivalent of beer. Under the latter conditions more bread is eaten, so that the beer is paid for twice, once in money and once in extra food containing the same carbon and hydrogen equivalents as the beer.

Liebig enters into the calculation of the oxidation of various foods in the body and gives the following values (p. 106):

100 Liters of O_2 combine with	And they warm liters of water from 0° to 37°
120.2 gm. starch	28.356
48.8 gm. fat	27.647

Liebig also calculates the caloric value of meat. He prepares a table of isodynamic equivalents which are given below, contrasted with the values given by Rubner (*d*) later in 1885 (p. 75).

Liebig writes:

Since the capacity of these substances (the respiratory materials) to develop heat through union with oxygen is dependent on the amount of combustible elements which equal weights contain, and since the amount of oxygen necessary for their combustion increases in the same proportion, therefore it is possible to calculate approximately their relative heat producing power or respiratory value. The following table contains the respiratory materials arranged in one possible order. The figures express the relative amount of each substance which a given amount of oxygen would convert into carbonic acid and water or

approximately how much one must eat in order to maintain the body temperature at a given level of metabolism during a given time:

Table of Isodynamic Values

	Liebig in 1846	Rubner in 1885
Fat	100	100
Starch	242	232
Cane-sugar	249	234
Dried meat	300	243

This, surely, is a divination of Rubner's subsequently enunciated isodynamic law.

As regards the oxygen requirement for the combustion of different foods, comparisons may be made between the findings of Liebig in 1846 and those of Loewy in 1911:

To oxidize	requires O_2 in c.c.	
	Liebig	Loewy
Fat, 1 gm	2050	2019
Starch, 1 gm	832	828

It is evident that Liebig clearly understood that it was protein, carbohydrate and fat which were oxidized in the body and that they were the source of energy and not carbon and hydrogen supposed to be produced from them.

Liebig divides the foodstuffs of man into two classes, the nitrogenous and the non-nitrogenous. The first class can be converted into blood; the other cannot be. The constituents of organs of the body are built up from those foods which are convertible into blood. In the state of normal health the other foodstuffs are used merely for maintaining the respiration process. He calls the nitrogen-containing foods the plastic foodstuffs and the non-nitrogenous, the respiratory foodstuffs. They are as follows:

Plastic Foods	*Respiratory Foods*
Plant fibrin	Fat
Vegetable albumin	Starch
Vegetable casein	Gum
Meat and blood of animals	Sugars
	Pectin
	Bassorin
	Beer
	Wine
	Brandy

"It is a fundamental fact, so far without a contradictory experiment, that the sulphur- and nitrogen-containing constituents of plants have the same chemical composition as the principal components of the blood. We know of no nitrogen-containing material of a composition different from fibrin, albumin and casein which is able to sustain life.

"The animal organism is certainly able to construct its membranes and cells, nerves and brain, the organic materials of ribs, cartilages and bones out of the constituents of its own blood, but these constituents must be already constructed in proper form or the production of blood and life itself is brought to an end.

"Looking at the matter from this standpoint, it is easily understood why gelatin is not a builder of blood or a supporter of life, for its composition is different from that of the fibrin and albumin of the blood."

Concerning the ultimate disposal of the products of metabolism, Liebig writes:

> The kidneys, skin and lungs cannot be the only ways through which products of the metabolism are eliminated from the body. The intestinal canal functions also as an organ of excretion and its relation to the respiration process must not be misunderstood.
>
> If the quantity of oxygen absorbed in a given unit of time is that which is exactly necessary to convert the products of metabolism present during the same period into carbonic acid, urea and water, then the intestinal canal will contain only indigestible substances.
>
> . . . In general it must be assumed that all of the nitrogen- and sulphur-containing constituents of the food of man are completely digestible, are brought into solution and absorbed into the circulating blood, for a property belonging to some parts must belong to all. In such cases it is undoubtedly true that the discovery of nitrogen-containing materials in the feces signifies that they can only be the products of the metabolism of the intestinal canal itself or products which have escaped normal metabolism and have been excreted from the blood by the intestinal wall.

Just before the publication of Liebig's great work Dumas, in glowing language, pictured similar interpretations without giving Liebig credit for the ideas. He utilized a formula similar to that given by Liebig without stating its derivation. Thus, in 1842, Dumas and Cahours presented the following penetrating conception:

> The food of an ordinary maintenance ration contains 16 to 21 gm. nitrogen. This nitrogen is almost entirely recoverable in the urine in the form of urea.
>
> Ignoring the intermediary phases, protein breaks up as follows:

$$C_{48}H_{37}N_{12}O_{15} + 100\,O = \begin{array}{lll} C_6\ H_{12}N_{12}O_6 & & \text{urea} \\ C_{42}\qquad O_{84} & & \text{carbon dioxid} \\ H_{25}\quad O_{25} & & \text{water} \\ \hline C_{48}H_{37}N_{12}O_{115} & & \end{array}$$

The only object in giving this formula is to enable one to calculate the heat of combustion of protein. Allowing for the daily production of urea from protein,

there would remain 50 gm. of carbon and 6 gm. of hydrogen suitable for oxidation; this would yield 575 calories. Since calculations based on the carbonic acid elimination and oxygen absorption show that a man produces between 2,500 and 3,000 calories daily, it follows that he needs an additional 200 gm. of carbon and 10 gm. of hydrogen to complete the required quantity of heat.

The writings of Dumas brought Liebig (*b*) to the defense of his priority in an article entitled, "Antwort auf Herrn Dumas' Rechtfertigung wegen eines Plagiats," published in 1842. He recited how, in the winter of 1840-41, he had lectured to his students upon: (1) the respiration process in its relation to the bile, (2) the nitrogen-containing substances of the vegetable kingdom are identical with those of the blood; and (3) sugar and starch are not food materials but serve for respiration and for fat production. A young Swiss student of Geneva came to Liebig with a letter from Dumas, attended the lectures, and afterward carried the information to Dumas in Paris. With volume 41 of Liebig's Annalen the name of Dumas as collaborator disappears from the front page. Berzelius sided with Dumas in this historic controversy, greatly increasing the bitterness of Liebig. The feeling between the two men, however, must have died down, for in a dedication to Dumas of his "Nouvelles lettres sur la chimie," dated Giessen, 1851, Liebig speaks in the most flattering terms of his old associate and brilliant antagonist.

Charges of plagiarism are contemporaneous with the progress of human thought. When two people work together they may find it possible to make the pleasing statement of Bidder and Schmidt, "As the result of mutual exchange of ideas and through intellectual metabolism, we find ourselves in entire agreement." But as regards the controversies regarding the priority of discoveries, such as grouped themselves around the person of Lavoisier and the person of Liebig, no such self-abnegation was possible.

Wöhler writes to Liebig regarding another matter in the following words (Moore, 1918):

> To make war upon Marchand (or any one else for that matter) is of no use. You merely consume yourself, get angry, and ruin your liver and your nerves—finally with Morrison's Pills. Imagine yourself in the year 1900, when we shall both have been decomposed again into carbonic acid, water and ammonia, and the lime of our bones belongs perhaps to the very dog who then dishonors our grave. Who then will care whether we lived at peace or in strife? Who then will know anything about your scientific controversies—of your sacrifices of health and peace for science? No one: but your good ideas, the new facts you have discovered, these, purified from all that is unessential, will be known and recognized in the remotest times. But how do I come to counsel the lion to eat sugar!

This is the correct interpretation to be placed upon rights of priority. The influence of an individual is evidently the result of the sum total of all activities of his life. If he contributes to the ideas of others, the

results may be of three kinds: (1) the donor may be publicly acknowledged; (2) the donor may be honestly forgotten and the recipient may honestly believe that he has for years held the same views; or (3) the donor may be well known to the recipient but be deliberately and systematically ignored. The last-named reaction is the one most difficult to bear with becoming humility of spirit, but, interpreted in the light of history, it signifies but little. It matters little to the world at large whether Bacon wrote Shakespeare or Shakespeare wrote it himself. The heritage of the masterpieces is what matters.

Before Liebig's death he wrote to Wöhler concerning the publication of their correspondence as follows: "When we are dead and gone these letters which united us in life will be as a token for the memory of man of a not frequent example of two men who, without jealousy or envy, strove in the same field and always remained intimately united in friendship."

Liebig's Munich Period.—In 1852, at the age of forty-nine, Liebig moved to Munich to become professor of chemistry there. His creative work ceased and a period of literary activity set in. He engaged in violent polemics with Pasteur, maintaining that alcoholic fermentation was a purely chemical phenomenon and not one of biological origin. He gave popular lectures in court circles and, with Richard Wagner, shared the popular adulation of the town. When Liebig's new gluten bread was put upon the market the townspeople stood in long lines before the bakeries to receive the precious product.

It may be of interest to pass here to the viewpoint of Liebig expressed in 1870 just before he died. In the interim the work of Bidder and Schmidt, of Bischoff and Voit, of Voit, and of Pettenkofer and Voit, had appeared, material which is still to be recorded.

Liebig writes as follows: "On the basis of general experience I formerly expressed the opinion that the source of mechanical work of the animal body must be sought in the metabolism, especially in the metabolism of the nitrogen-containing constituents of muscle. The capacity for work in two individuals would therefore depend upon their respective mass of muscle tissue, and the endurance of each would depend on his capacity to rebuild the broken-down muscle substance from the inflowing food material.

"It is well known that hard-working men eat much meat. An employee (Bräuknecht) in Seldmeyer's large beer brewery consumes daily 810 gm. of meat, 600 gm. of bread and 8 liters of beer. One should be cautious in adopting the popular Bavarian idea that it is the beer which gives muscular power, for the beer drinkers are also the greatest consumers of meat.

The question regarding the source of muscle power has been confused through a conclusion which has been shown to be false and for which I am to

blame. It was an error to assume that, if urea were an end-product of the oxidative metabolism of muscle, then one could measure the intensity of the work done by the quantity of urea in the urine.

The first facts contradicting the idea that urea is a measure of muscular activity were communicated by Bischoff and by Bischoff and Voit of Munich, which researches are to be considered as the extension of work accomplished in Giessen. It is hardly necessary to state that these experiments always excited my keenest interest because they were effected with my method of urea determination. . . .

These experiments firmly establish the fact that, although urea elimination is a measure of protein ingestion and metabolism, it is not a measure of the work done by the body.

When one thinks these matters over it is apparent that the facts could not be otherwise. For if the metabolism of the muscle increased with work a man could exhaust his entire supply of muscle tissue, because work is directed by the will.

He criticizes Frankland's comparison of the muscle with a steam engine, as follows:

It is certain that the wonderful structure of the animal body and of its parts will long and perhaps forever remain an insoluble riddle. But the processes within the organs are of chemical and physical nature, and it is incomprehensible that oxygen and combustible materials are under the control of nerves to induce their union. The factor of voluntary nerves upon muscle activity must be of a different order. . . .

I consider that those investigators who have busied themselves with the question of the source of muscular power have thought its solution too simple and that it will be many years before a proper viewpoint leads to clarity in the solution of this subject. I have no desire to enter into the dispute.

Liebig discusses the activity of the yeast cell as follows:

A close consideration of the behavior of the yeast cell may be desirable in order to give a more definite idea of what transpires in living muscle.

It is certain that motions occur within the yeast cell through which it is enabled to accomplish external work. This work consists in the cleavage of carbohydrates and similar substances. This is chemical work; it would be mechanical work if the yeast were able to split wood, which is likewise carbohydrate.

One part of yeast can destroy sixty parts of its weight in sugar, according to Pasteur. A gram of yeast can produce the heat equivalent of 148,960 gram meters of work without the intervention of oxygen.

The cause of all these activities lies in the motions of the contents of the yeast cells.

In similar manner the motions of life are present in muscle cells, without muscular contraction resulting. When the movement within the muscle cells rises above a certain limit, muscular contraction follows.

Liebig enters into a defense of the use of Liebig's extract of meat. At one time he had regarded it, when mixed with potatoes, as the equivalent of meat. He quotes *Hippocrates:*

"Soup and pap were discovered because experience has taught mankind that foods which are good for healthy people are not good for the sick."

One need only compare the capacity for work of the German workman, who lives on bread and potatoes, with the English or American workman, who eats meat, in order to gain a clear insight into the importance of the kind of food taken. The partaking of meat raises the capacity, the power and the endurance for work. Or compare an English statesman who may speak for five hours or more in a Parliamentary debate, and who in the full possession of youth may still engage in a strenuous hunt at the age of sixty, with a German professor of the same age who sparingly conserves the rest of his physical powers and who is exhausted by a walk of a few hours.

Liebig cannot understand the modern expressions, "organized protein" and "circulating protein"; they confuse him to such a degree that he cannot tell his right hand from his left.

It is right to investigate a single phase in order to comprehend the existence and activity of a whole process, but in order to interpret correctly the results of investigations one must have a clear picture of the manifold phenomena and the limitations affecting the entire problem.

I have a general knowledge (Ich weiss so ziemlich) of how to estimate the importance of experiments and facts, and of their inequality as far as drawing conclusions is concerned. The simple observation of a natural phenomenon arranged without our assistance is more important and often much more difficult than the phenomena observed in an experiment produced by our will. In the first reality is mirrored, while an experiment represents the imperfection of our understanding.

I remember that many years ago during a walk between Berchtesgaden and the Königssee, a very simple observation led me to the conclusion of the source of carbon in plants. At that time there was great confusion in the subject, and it was difficult to exclude humus from consideration as a factor. But on this walk Nature gave the proof that the carbon of the plant could arise only from carbonic acid. For one finds rocks there which had been dislodged and had fallen from the higher mountain side, and trees thirty or forty feet high grow on the rocks, sending their roots between the crevices while the rocks are covered only with moss and a layer of dust. It was impossible to conceive that humus could have conveyed carbon to vegetation of this sort.

Similar observations can be made in the laws of nutrition if one has but the good-will to see them.

It appears to me to be almost unthinkable that the high value placed by the French family upon their "Pot-au-feu" is merely based on custom; or that one of the greatest military physicians of the French army, Dr. Baudens (Baudens, 1857) would dare to say "La soupe fait le soldat" unless he was absolutely convinced of the high potency of meat soup containing the necessary vegetables which the French soldier often prefers to meat.

Liebig laments the criticism of his extract of beef and quotes Goethe, "The word of a wise man teaches me that if a person once does a thing which is good for the world, the world takes pains to see that that person does not do it a second time."

One may annotate Liebig's opinion of Voit's "circulating protein" and "organized protein" by citing a letter which Liebig wrote to Wöhler

in 1870, in which he says that he is considering giving up his lectures during the summer semester upon the subject of animal chemistry and nutrition and continues, "I find so little to interest me in what others are doing in this subject I lose all desire to take part in it. They perform nothing but small experiments which lead to nothing. Modern physiologists lack a great idea upon which all investigations depend."

Wilhelm Ostwald comments that this is the usual experience of parents with their children, and is the greater the more capable and important the children become.

It may be of interest in this connection that I heard Voit tell my father in 1891 that there were no young, promising physiologists of about forty in Germany at that date, a generalization which would have included Rubner (born 1854), Kossel (born 1853) and Hofmeister (born 1850).

The happy ideas obtained as the result of Liebig's walk between Berchtesgaden and the Königssee recalls the statement made by Helmholtz at a festival given in honor of his seventieth birthday, in which he told that he had never had a great thought come to him at his desk nor when he was tired nor after taking a glass of wine, but usually when he was walking in the garden thinking of other things.

All the quotations of Liebig's later views are from writings published in the year of the Franco-Prussian War of 1870. In his "Thierchemie" of 1840 and in several other of his publications at that period occur the following memorable words: "Culture is the economy of power, the sciences teach how to produce the greatest results by the simplest means with the least expenditure of energy. Every unnecessary use of energy, every waste of power in agriculture, industry, science, or in statecraft is characteristic of crudeness or lack of culture."

Concerning the results of the conflict of 1870, Liebig moralized as follows: "It was a battle between knowledge and science on one side and empiricism and routine on the other, in which, as in agriculture, knowledge won."

Hear this realizing cry of Pasteur (Vallery-Radot, 1902) which followed the defeat of France in 1870 concerning the "forgetfulness, disdain even, that France had had for great intellectual men, especially in the realm of exact science." He says, "Whilst Germany was multiplying her universities, establishing between them the most salutary emulation, bestowing honors and consideration on the masters and the doctors, creating vast laboratories amply supplied with the most perfect instruments, France, enervated by revolutions, ever vainly seeking the best form of government, was giving but careless attention to her establishments for higher education.

"The cultivation of science in its highest expression is perhaps even more necessary to the moral condition of a nation than to its material prosperity."

Nor was the development of German science ignored in England, for Matthew Arnold wrote in 1868: "Petty towns have a university whose teaching is famous throughout Europe, and the King of Prussia and Count Bismarck resist the loss of a great savant from Prussia as they would resist a political check."

Let us not forget the environmental conditions under which men like Liebig may be fostered and developed.

Bidder, F. W. (1810-1894) and Schmidt, C. (born 1822).—In order to complete the story of Liebig's life this history has been diverted from its chronological sequence, and it is now necessary to tell of the activity of the period essentially coincident with the date of the publications of Regnault and Reiset. At the same time that these men were at work in Paris, Bidder and Schmidt (*a*) were active in the German university established at Dorpat in Russia. In 1852 they published their book, "Die Verdauungssäfte und der Stoffwechsel." Voit often referred to this book as a veritable mine of information. The book, however, has never been as well known as it should be. The statement still found in textbooks on physiology that the influence of food upon the bile flow has never been investigated finds its refutation in this volume, published in the middle of the last century. Here, also, one finds the method of computing the metabolism used by those who employed the Pettenkofer-Voit respiration apparatus.

Bidder and Schmidt were much more profoundly influenced by the doctrines of Liebig than were Regnault and Reiset. Had the methods of the four investigators been combined, much of value would probably have been rapidly uncovered. But Reiset's publication of 1868 on the metabolism of farm animals shows no knowledge of the publication of Bidder and Schmidt. To promote science one must know of contemporaneous activities in many lands, as well as of the older historical happenings.

C. Schmidt, who had been a pupil of Liebig and Wöhler, began work six years before (1845) the completion of the combined work of Bidder and Schmidt. Schmidt had planned an experimental critique of the metabolism of the higher vertebrates. His idea was to study in a few typical forms the following main factors: oxygen absorption, carbonic acid and urea elimination and the energy statistics of fasting animals, accomplished upon the same individual under identical conditions. Having accumulated this mass of observations concerning the typical intensity of the respiration and the protein consumption on the more prominent types of vertebrates, it was planned to investigate in similar fashion the size of the intermediary metabolism, the effect of external temperature and the effect of partaking of protein, fat and carbohydrate, and then to reduce the sum total of all the observations to a systematic whole.

It was beyond the power of a single individual to accomplish this plan. A preliminary investigation established the specificity of the

enzymes, that yeast can act only on sugar and produces only alcohol and carbonic acid, emulsin acts only on amygdalin, converting it into hydrocyanic acid, benzyaldehyd and sugar; the same principle follows as regards the digestive enzymes. The determination of the characteristic metabolism, including the respiratory exchange, the analysis of urine and feces and record of the body temperature upon a single animal, each observation continuing over several weeks, required such unremitting attention by a single observer that even one provided with a powerful constitution found it almost beyond his power of accomplishment.

Bidder, who had become interested in the lymph flow as a possible measure of the intermediary metabolism, united his work to that of Schmidt and they decided to work together. Bidder edited the part about the digestive juices and Schmidt that about the metabolism and, "as the result of mutual exchange of ideas and intellectual metabolism, we are in entire agreement."

The intermediary metabolism is practically *terra incognita.* To investigate this the authors seek especially to determine the bile excretion in relation to the total ingesta and excreta of the body.

They ask, "Is bile an excrement or not?" Schwann first described bile fistulæ. In at least six of his dogs the cause of their death could have been attributed only to the removal of the bile (1844).

Blondlot disputed as to this being the cause of death (1846). They note that the bile solids eliminated daily constitute a three-hundredth part of the solids of the body and they inquire into the question of the quantity of bile reabsorbed by the intestine, as follows: "We investigated the content of bile in the feces of a dog weighing 8 kg. during a five-day period. In order to obtain exactly the quantity of feces belonging to this period the animal was given only meat during the experimental period, and before and after the experiment he received a diet of "Schwartzbrod," which yields an extraordinarily voluminous feces, greatly resembling the bread itself and therefore easily recognizable. The fecal material between these two portions must have been derived from the meat diet or from the residues of the bile excreted into the intestine."

The feces following meat ingestion weighed 97.3 gm. and contained 40.9 gm. of dry solids. "Since this fecal matter contained only traces of bile constituents, and since the quantity of bile solids flowing into the intestine must have aggregated 39.52 gm. or nearly the quantity of the entire feces, it necessarily follows that the larger part of the bile must have been reabsorbed. Still more convincing is the fact that 39.5 gm. of bile solids must have contained 2.37 gm. of sulphur, whereas the entire sulphur content of the feces was only 0.384 gm., more than half of which must have been derived from hair, for, excluding the hair in the feces, only 0.154 gm. of sulphur were found. Almost all the biliary sulphur must have been absorbed into the blood and we are therefore convinced

that the larger part, perhaps seven-eighths of the biliary solids return to the blood and undergo further metabolic transformations before they are removed from the body by other channels."

When Bidder and Schmidt operated on about a dozen cats by the method of Schwann they all died of peritonitis in two or three days, but in dogs only two of eleven died of peritonitis.

Liebig had stated that the bile was reabsorbed and was used as a "respiration stuff." It was formed in the body and then later, when reabsorbed, was oxidized to carbon dioxid, being an example of the steps in the metamorphosis of organic substance during life. To what an extent does this process take place?

A cat excreted 0.6 gm. bile containing 0.033 gm. solids per kilogram of animal in the third hour after a meal.

There was no increase in the flow of the bile after giving fat. The quantity was the same as that after 48 hours fasting. But the ingestion of meat increased the volume of the flow and the weight of the solid constituents.

In dogs with bile fistulæ, the secretion of the bile cannot be very far from normal because of the complete digestion of the foodstuffs, of the effect of these upon the bile flow and of the perfectly normal condition of the liver and its vascular supply.

This fate of the bile does not exclude its having certain functions, while it is present in the gastro-intestinal tract. They can confirm the recent work of Hoffmann regarding the antiseptic action of the bile on the intestinal contents. For they observed that dogs whose bile is conducted away through a fistula pass feces which have an extremely foul, almost carrion-like, odor, and that there is flatulence induced by a gas of evil odor. However, when bread alone was given the feces and fecal gas had no odor.

Much more important is the question whether the bile has a digestive action in making materials more fluid. When meat is given to dogs with biliary fistulæ, it is perfectly digested and absorbed and no particles of undigested meat can be microscopically detected in the dog's feces. This was true even when large quantities of meat were given. However, when 113.6 gm. of fat were ingested, 72.2 gm. of fat substances appeared in the feces. When black or white bread was given no starch granules were present in the feces and the dog even gained weight. But when fat was given there was very poor absorption; in one case only one-tenth was absorbed. Hence a normal dog absorbs much more fat than one with a bile fistula.

They find, also, that there is much less fat in the chyle of the thoracic duct of a dog which had been provided with a biliary fistula than in that of a normal animal. The action of the bile is evidently upon fat or upon the absorbing intestinal surface.

Neutral fat in a melted state penetrates the epithelia of the intestinal wall provided the same is covered with bile in a living animal, whereas it is impermeable to fat when it is not covered with bile. There is a greater attraction for fat in the former case. If two capillary tubes be taken and one be soaked in fresh bile, the other in water or normal saline, and then both be dipped in oil, the fat will rise much higher in the tube dipped in bile than in the other tube (we moderns would call this a diminution of the surface tension).

They state that when the bile is drawn off through a biliary fistula there is an increased intake of other food to compensate for the losses through the bile.

Is the absorbed bile eliminated through the kidneys or through the lungs? The nitrogen content is too small to contribute much to the nitrogen content of the urine, and hence Liebig concluded that bile was a respiratory material (material fit for respiration), yielding carbon dioxid and water as end products. Certainly, all the carbon of the respiration does not have to pass through the bile prior to oxidation, for the total bile contains only 0.5 gm. of carbon, the expired air 8.6 gm. of carbon per kilogram of body weight in the dog in twenty-four hours. However, the 0.035 gm. of nitrogen eliminated in the bile per kilogram of body weight might readily be that quantity which was liberated as free nitrogen and was expired in the respiration.

Bidder and Schmidt describe what is now known as "basal metabolism," as follows: "For every species of animal there is a *typical minimum* of necessary metabolism which is apparent in experiments when no food is given (im nüchternen Zustande). The excess over and above this necessary measure of typical metabolism can be termed *luxury consumption,* although the well-being and the energy of all the functions of life are considerably increased through this increased activity of metabolism."

Bidder and Schmidt now attempt the first computation of the total metabolism, as calculated from the respiratory as well as from the urinary and fecal pathways of elimination. They say, "To give the total figures would involve too much printing." The following was an experiment of June, 1847, accomplished on a pregnant dog.

In the first place they give the following elementary analysis of dry meat free from ash:

C	53.01	per cent
H	7.02	
N	16.11	
O	22.86	
S	1.00	
	100.00	

During an eight-day period they give to a dog 1866.7 gm. of meat of the above-mentioned constitution, together with 27.4 gm. of fat. In the urine and feces of this period they find 62.36 gm. of nitrogen, which would correspond to a destruction of 387.09 gm. of dry flesh or 1695.5 gm. of living tissue of the dog.

The balance would therefore read:

	Grams
Flesh destroyed	1695.5
Flesh ingested	1866.7
Flesh retained	171.2
Add fat retained	27.4
Total maximum assimilation	198.6

The gain in body weight was 337 gm., the excess was attributable to water retention.

Not only was the elementary composition of the urine and feces determined (as in the method of Boussingault), but on seven different occasions the carbon dioxid in the respiration was determined in periods lasting one hour each. After this fashion Bidder and Schmidt were able to estimate the quantity of the carbon metabolism, which they express as follows:

	C in grams
387.09 gm. of muscle metabolized contain	205.20
In the excreta were eliminated	194.02
Retained in the body	11.08

Since the total carbon elimination in the urine, feces and respiration was less than that derivable from the flesh metabolized, it was evident that the ingested fat could not have participated in the metabolic process, but must have been absorbed and stored in the body. Analysis of the feces showed the almost complete absorption of fat.

This method of determining the total metabolism is in principle that used by Pettenkofer and Voit a decade later.

The authors strike the following balance, showing the fate of 100 gm. of meat protein:

	C	H	N	O	S
100 gm. meat protein	53.01	7.02	16.11	22.86	1.00
In 34.52 gm. urea	6.91	2.30	16.11	9.20	
In 65.48 gm. rest for respiration and bile production	46.10	4.72	—	13.66	1.00

A very small quantity of carbon, hydrogen and oxygen (3 to 5 per cent) and a lesser portion of the sulphur as sulphid of iron were eliminated in the feces, but the greater portion of the sulphur was eliminated in the urine in the form of sulphuric acid.

From the data available they calculate the oxygen necessary for the oxidation of the materials metabolized by the dog. They note that Regnault and Reiset obtained a relatively greater volume of oxygen absorbed than volume of carbon dioxid given off and attribute this to the fasting condition of the animals, since fat contains relatively more hydrogen than protein and therefore more water was produced at the expense of oxygen absorbed than in the case of a protein diet. Bidder and Schmidt estimate the respiratory quotient of a meat-fed dog to be 0.84.

They further estimate that five per cent of the total carbonic acid expired passes through a stage of intermediary metabolism by way of the bile.

In a fasting cat Bidder and Schmidt determined daily for eighteen days the water eliminated in the urine and feces, the urea, sulphuric and phosphoric acids in the urine, the expired carbonic acid and (for ten days) the dried solids of the bile. From the nitrogen excreted they calculated the quantity of carbon attributable to the protein metabolism of the time. Subtracting this protein carbon elimination from the total carbon elimination in the urine, feces and respiration, they were able to calculate the quota of respiratory carbon attributable to fat metabolism and from this the quantity of fat metabolized during the fasting period. This is again the method followed by Pettenkofer and Voit.

They make the following table to represent the starvation period (eighteen days):

From the metabolism of	C	H	N	O	S	P_2O_5
204.43 gm. protein ...	102.24	13.43	30.81	43.81		
132.75 gm. fat	103.72	15.59		13.45		
Total	205.96	29.02	30.81	57.26	2.167	3.761
Excreted by lungs, urine and feces	205.96	4.67	30.81	18.42	1.127	3.565
Rest (to be expired as water)		24.35		38.84		

	O_2 Gm.
190.78 gm. expired C require to produce CO_2...	508.74
24.347 gm. " H " " " H_2O ..	194.78
	703.52
Less O_2 contained in the products of metabolism....	38.84
Oxygen which must have been used.....	664.68

What one now calls the "respiratory quotient" was 0.765, whereas Regnault and Reiset had found 0.744.

After this fashion the metabolism was also estimated for each day. The oxygen consumption fell from 44 gm. on the second day to 31 gm. on the sixteenth day, just before the premortal fall in body temperature.

At the death of the animal the body was sectioned and the various parts were weighed when fresh and their dry weights and fat contents were later obtained. A normal cat was then killed and similarly analyzed. The first cat before fasting had weighed 2572 gm., and at death 1241.2 gm. The original composition of the organs of the cat, when it began to fast, was computed on the basis of the analysis of the normal cat. The loss of weight of different organs in starvation could then be computed.

This is the historical forerunner of several similar extremely laborious experiments.

In 1852 we might have read this modern statement:

> The extent of the respiration, like every other component of the metabolism process, is to be regarded as a function of one variable, the food taken, and one constant, a distinctly typical metabolism (Respirationsgrösse) which varies with the age and sex of the individual. This factor characterizes every animal of a given race, size, age and sex. It is just as constant and characteristic as the anatomical structure and the corresponding mechanical arrangements of the body. It is in the main determined by the heat consumption in the organism; that is to say, the replacement quota for heat lost to the body through radiation and conduction to the environment in a given unit of time. It may therefore be used to determine this, or in case the factor of heat loss is known, one can deduce the extent of the metabolism.
>
> This typical metabolism . . . is that of the fasting animal. It must be nearly the same in animals having the same body volume, surface and temperature; the larger the body surface, the body volume and temperature remaining constant, or the higher the body temperature with surface and volume constant, the higher will be the metabolism as determined by the laws of static heat.
>
> Of course a sharp mathematical treatment of this phenomenon can be thought of only after very numerous and exact experimental determination upon animals of most varied form, size and temperature.

A footnote states: "This is an extensive program and may require many decades for its solution." It is suggested that experimenters divide the investigations into the animal kingdom after the fashion that astronomers have divided portions of the heavens among themselves for observations. Bidder and Schmidt state that, acting with this intent, they have dealt almost exclusively with the cat.

"Animals cannot maintain the typical metabolism over a prolonged fasting period."

They define a *"typical food minimum"* as that quantity of assimilable food upon which the body maintains its weight over a long period of time. A slightly lesser quantity than this causes the body to lose weight.

After giving much meat "there is a double Luxus consumption: ex-

pressed (1) by excessive oxidation, heat production, by increased evaporation of water, and (2) by the cleavage of one-eighth of the carbon and one-third of the hydrogen of protein in the form of urea. Only the smallest quantity of this urea production is necessary for the maintenance of the animal; it arises from the cleavage of the metabolized body protein itself. The larger part is eliminated in order to yield the carbon, hydrogen and oxygen containing rest in a form suitable for respiration and not injurious to the body. Protein nitrogen cannot be eliminated through the lungs, for nitrogen scarcely combines with blood and if liberated would fill the capillaries with gas, nor can ammonia be produced for this destroys the blood corpuscles."

The greater the quantity of fat given, the smaller is the Luxus consumption in carnivora. Among herbivora it is usually very slight because here protein is taken in conjunction with an excessive quantity of carbohydrates and is almost entirely used in replacement (Wiederersatz) of the body protein necessarily destroyed—which latter is the typical (minimum) protein metabolism.

They find that following fat ingestion the feces contain magnesium and calcium soaps, as shown by Boussingault.

The authors suggest that protein may be composed of taurin, glycocoll and a carbohydrate, a "respirations rest," they call it. One hundred grams of protein would contain:

Taurin	6.2 gm.
Glycocoll	79.3 gm.
"Respirations rest"	28.3 gm.

Taurin and glycocoll would yield 33.2 gm. of urea and 49.8 gm. of carbohydrate.

They add, "It is not possible to formulate a well-grounded hypothesis concerning the formation of urea because of the present uncertainty of our knowledge of the composition of protein."

At the end of the book there is a beautiful chart showing the metabolism of the fasting cat and giving the bile secretion as intermediary metabolism.

Max von Pettenkofer (1818-1901).—Pettenkofer, who is well known as the man who first raised hygiene into a science of sufficient dignity to be provided with an independent laboratory of its own, was not only responsible for the modern drainage system of the town of Munich, which converted it from the "pestilential city of Europe" into one which was extraordinarily healthful, but he also made notable contributions to the physiology of nutrition.

He noted that a child with St. Vitus' dance, who partook of an inordinate amount of apple parings, voided a urine containing a large amount of hippuric acid. This was one of the earliest discoveries of the influence of food on the composition of the urine.

The celebrated Pettenkofer reaction for bile salts was not determined by accident. Liebig thought that fat arose from carbohydrate. To test this, Pettenkofer treated a solution of cane-sugar with strong sulphuric acid in order to dehydrate the sugar and obtain a rest rich in carbon which might be convertible into fat. Since the liver or bile was believed to further such a reaction, Pettenkofer added bile salts to the mixture and obtained, not fat, but the well-known color reaction. Using this reaction, he was able to show that normal feces contained no bile salts, though these might be found in diarrhea.

In 1844 Pettenkofer found a compound in the urine which united with zinc chlorid and he established its chemical composition. Its identity remained hidden until it was one day shown to Liebig, who warmed it over a flame on a porcelain cover, and from the odor evolved immediately concluded that it must be related to the creatin of muscle. Such is genius!

Voit, who was acquainted with the work of Bidder and Schmidt, suggested to Pettenkofer that he devise a respiration apparatus which would measure the output of carbonic acid and water in a dog weighing 20 to 30 kilograms. Pettenkofer, who was interested to work with men as well as with dogs, constructed the chamber of the apparatus so that it had the size of a moderately large stateroom on a steamer, in which a man could sleep, work and eat without discomfort. The ventilation of the chamber was about 500,000 liters daily. Portions of the ingoing air and portions of the outgoing air were diverted in their course and analyzed for carbon dioxid and water. The increase in these materials in the total air leaving the chamber represented the amounts given off by the subject of the experiment. This was the first respiration apparatus checked by burning a candle in it. Pettenkofer criticized Regnault and Reiset for not doing this, and thus establishing the limitations of the accuracy of their work, a test which would have shown why nitrogen gas was apparently at times absorbed and at other times excreted by their animals.

Voit writes: "Pettenkofer's talents produced the respiration apparatus and after that we together began experiments with it. Pettenkofer and I had an equal share in the experiments."

Carl von Voit (1831-1908) was born in Amberg and was the son of August Voit, architect of the Munich Glaspalast. In 1848 he went to Munich to enter the university. He joined a students' *corps* but soon left it in disgust, feeling it was no place for him and perhaps reflecting upon the German witticism, "Er war so dumm dass selbst seine eigene Corpsbrüdern es bemerckt haben." He entered enthusiastically into the republican ideas prevalent in that year in Germany. His revolutionary activities earned him a black mark on the qualifications list of the university, a fact which he discovered long afterward when he had risen in position and fame.

After passing his "physicum" examination, he went to Würzburg in 1851, which was at that time a much more celebrated medical center than Munich. After a year he returned to Munich, which had received an academic stimulus by the arrival of Liebig. He graduated in medicine in 1854 and, in order to prepare himself for a scientific career, he devoted the following year to attending lectures in physics, zoölogy, anatomy and chemistry. The last-named course was given by Liebig. He entered the laboratory of practical chemistry then conducted by Pettenkofer. With Pettenkofer he studied an outbreak of cholera, especially the accumulation of urea within the organism during the infection and its elimination subsequently. He devoted a large part of his time to the study of the works of the great Liebig, whose reputation filled the world. On Liebig's advice he spent a year with Wöhler in Göttingen. He then planned to pass a year with Bidder and Schmidt in Dorpat, but he was turned from this by an offer of an assistantship to Bischoff, professor of anatomy and physiology in Munich. In 1859 he became professor extraordinarius, and in 1863, at the age of thirty-two, professor ordinarius of physiology in Munich, a position which he held for forty-five years until his death.

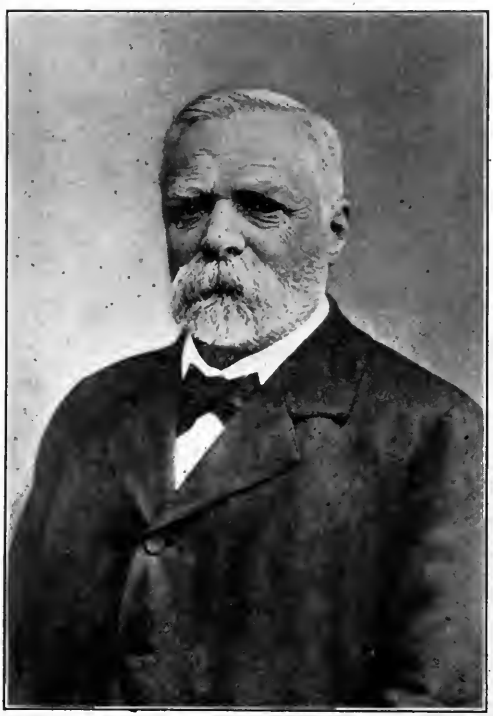


Fig. 7. Carl Voit. From a plate in the "Jubelband" of the "Zeitschrift für Biologie" (Vol. XLII), published in honor of his seventieth birthday.

During his early student days he had a desk adjoining that of Brush, for many years the dean of the Sheffield Scientific School. Of him Voit said, "I can see him now, how accurately he worked!" And throughout Voit's life it was "die Genauigkeit" upon which he placed the maximum of stress.

Perhaps it may be of interest to present some of the earliest of Voit's work in this historical review. The ideas are largely expressed in the light of the doctrines of Liebig. A young man is usually at first imbued with the doctrines of his master. The master who has a knowledge of accumulated facts can often most helpfully attempt to give the reasons

why things are; in other words, the doctrines and the theories. It is only later, when the young man has accumulated new facts out of harmony with the old theories, that those theories are overthrown and left as wrecks by the wayside. That is the history of science.

Voit (*b*) has put the matter thus:

> I cannot agree with those who think that because they do not agree with our conclusions they can overthrow the whole piece of work (that of Bischoff and Voit). For even though our theories should be as bad as represented, the important part of the work, the experimental results, would still remain. Those who know the history of science should have no idle illusions over the value of their own opinions. Upon every page of history one can read that the results of a properly devised experiment are immortal, whereas the theories drawn from the observation are frequently shown to be wrong, because it was not possible at the time to take into consideration all the factors at work during the experiment.
>
> . . . From theories further scientific progress is evolved, they stimulate renewed activity. It often happens to the investigator that others with little trouble to themselves present new conceptions of the work accomplished by himself, but the intelligent man, whose opinion and not that of the world in general is worth while, will not forget to whom credit for the service belongs.

An early work by Voit, "Beiträge zum Kreislauf des Stickstoffs" may be thus abstracted: In recent times one has sought to obtain a more intimate knowledge of the metabolism in the animal body by comparing the intake of various constituents of food with the constituents of the outgoing substances. In this category belong the experiments of Bidder and Schmidt and of Bischoff (1853).

Bidder and Schmidt found in cats and dogs that almost all the nitrogen was eliminated in the form of urea. In one cat fed with meat 99.1 per cent of the ingested nitrogen was found in the urine, 0.2 per cent in the feces, leaving only 0.7 per cent for the respiration.

Barral taught from experiments on himself that 8.33 per cent of the ingested nitrogen was eliminated in the feces, 42.07 per cent in the urine, leaving over 50 per cent for elimination by the lungs, an amount certainly too large in the light of recent exact determinations of the nitrogen elimination in the respiration, especially in those of Regnault and Reiset, who never found more than 1/50 and usually less than 1/200 part of the ingested nitrogen thus eliminated. Voit calculates that Regnault and Reiset's dogs, which absorbed between 121 and 212 gm. of oxygen daily, could have eliminated only between 0.04 to 3.69 gm. of nitrogen gas in twenty-four hours.

Both Lehmann and Boussingault, working with indirect methods, found that much of the ingested protein nitrogen must have been eliminated in the urine.

Bischoff was the first to use the titration method of Liebig for the determination of nitrogen in the urine. This method is exceedingly accu-

rate and rapid. Bischoff could not find all the ingested nitrogen in the urine and feces. (The urines, however, were frequently alkaline.) When 500 gm. of meat were given to dogs a third of the nitrogen content, or 6 gm. must have been eliminated in the respiration. As this contradicted Regnault and Reiset, Bischoff concluded that the nitrogen was probably expired in the form of ammonia.

Perhaps Liebig's titration method might be wrong, so Voit devised a method of distilling the ammonia derived from urine dropped upon soda-lime. He made fifteen comparative tests, the first of which is thus recorded:

	N content of 5 c.c. urine in grams
Liebig's method	0.2380170
Soda lime method	0.2277660

(The accuracy of this method of checking the results was subsequently tested by Liebig himself and found to be correct.)

Neither Bidder and Schmidt, nor Bischoff, nor Voit, ever observed undigested meat in the feces of a dog. But the dry feces contained 6.41 and 6.52 per cent of nitrogen.

Voit finds meat contains varying amounts of water and of nitrogen, the latter between 3.41 and 3.69, with an average of 3.59 per cent. Therefore, one cannot tell the exact composition of meat without some degree of error.

Forty kilograms of meat, if estimated at 3.4 per cent of nitrogen and then at 3.5 per cent of nitrogen content, would mean a variation of 40 gm. of nitrogen.

Voit adopts the value 3.4 per cent. of nitrogen and he chooses well-selected whole pieces of lean meat for his experiments in feeding animals.

He always collects the urine freshly voided from a trained dog and the urine is always acid.

In this early work Voit gave to a dog weighing 27 kg. 1500 gm. of meat for four days and collected the nitrogen eliminated in the urine, feces and the bile. The dog lost 255 gm. in weight (this multiplied by 3.4 was believed to give the contribution of body protoplasm to the nitrogen excreted). The nitrogen balance read as follows:

	Grams		Grams
N in meat	204.00	N in urine	197.48
N in lost body weight	8.67	N in feces	8.65
		N in bile	2.09
	212.67		208.22

In another experiment, using a normal dog, the intake of nitrogen contained in protein was 180.52 gm. and the outgo 180.96. In three of the five experiments the whole of the ingested nitrogen in meat was recovered in the urine and feces. This did not support the idea that protein nitrogen is eliminated in gaseous form through the lungs and the skin.

Bischoff stated that a part of the protein metabolism must be used for the growth of the hair and the epidermis, and this would still further lessen the possibility of its elimination as a gas in the experiments as computed by Voit.

This work of Voit was carried further and published by Bischoff (born 1807) and Voit (*f*) in 1860 under the title, "Die Gesetze der Ernährung des Fleischfressers," of which the following is an abstract:

"We propose to consider nutrition and the energy relations therein involved as they concern the animal organism, much of which may seem to be theoretical and therefore of little importance but which really embodies the sum of the recently acquired knowledge concerning energy and matter and which in part is concerned with our own observations."

All the experiments were done by Dr. Voit with the assistance of a laboratory servant and it is Dr. Bischoff's opinion "that the numberless analyses, the combustions and nitrogen determinations of various foods, of feces, etc., could not have been done with greater care or more tireless zeal than they were done by Dr. Voit."

They do not believe that all the protein of the ingesta must first be organized into the material of living cells before it can be metabolized, but rather that the fluid protein of the blood penetrates living cells there to be destroyed.

A dog was given 250, 500, 800, 1000 gm. of meat and still lost body nitrogen. With 1800 gm. of meat the urea nitrogen was equal to that of the food and when 2000 and 2500 gm. of meat were given the dog added flesh to his body, but this had hardly begun before the quantity of urea increased in the urine because the mass of metabolizing body tissue had been increased. The dog would not eat more than 2500 gm. of meat.

The methods of calculation of the metabolism used by Bischoff and Voit were much more crude than those of Bidder and Schmidt who preceded them. But the records of the protein metabolism, as measured in the nitrogen in the meat ingested and in that of the urine and the feces, are the classical observations on the subject.

In one experiment a dog weighing 35 kg. was given 31.6 kg. of rye bread during a period of 41 days. The animal received 405.29 gm. of nitrogen in the bread and eliminated 531.67 gm. in the urine and feces, indicating a loss of body nitrogen of 126.38 gm., which corresponded to a loss of "flesh" amounting to 3717 gm. Though the food was evidently insufficient, the dog appeared well nourished and active at the end of the experiment. His actual loss in body weight was only 690 gm. during

the period. This was because of the saturation of the body tissues with water when taking the bread diet, for when he was given 1800 gm. of meat he passed a great stream of water, losing 300 gm. in body weight in spite of a retention of the protein of meat which would have been the equivalent of an addition to the body of 600 gm. of new "flesh" (*vide* experiment of Stark, p. 14).

The authors found that, though gelatin could spare some body protein, it could not entirely prevent its loss. They state that it is an incomplete (ungenügendes) foodstuff.

Results—briefly abstracted.

We hold it for proved that the continued power to maintain movement on the part of a fasting organism is derived from the metabolism of protein.

The three factors which induce metabolism are "blood, organ and oxygen," and we believe that the metabolism of an organ is brought about by the united action of all three influences.

The mass of non-nitrogenous and nitrogen-containing tissue, the quantity of blood and blood plasma and the amount of available oxygen, these three factors determine the height of the metabolism.

If one gives to a fasting dog meat in such quantity that a loss from the dog's body is not prevented, the metabolism rises. The increased quantity of blood plasma increases the metabolism, although the mass of the organs remains the same; the influence of oxygen, on account of the increased food and metabolism, is greatly reduced. . . . As oxygen is present only in limited amount, its action is reduced upon both body protein and body fat; the metabolism of these is in consequence reduced.

If we increase the food protein and the blood plasma, the metabolism is constantly increased until we reach a point when loss from the body is equal to its repair. This is the moment when the metabolism of the protein parts of the organism has so increased as to acquire all the oxygen available, and the metabolism of fat ceases.

If the amount of food be still further increased the metabolism scarcely increases, for the available oxygen, through union with metabolic products, has been reduced to a minimum. This is the moment when deposit, increase in mass, excess for reparation, must and can ensue. . . .

But this process has a limit. As the intake of meat and the mass of the nitrogen-containing tissue increases, the metabolic products also increase. These require more oxygen. But the action of this is so reduced that, in spite of the increased bulk of the plasma and of the organs, a limit to the metabolism is set. As soon as the limit of metabolism is reached the limit of energy production is also reached. If energy is no longer present and available, it is also no longer possible to increase the metabolism. The animal can no longer eat and refuses food. With a limitation of food intake the volume of blood and plasma falls and the former condition returns.

This process constitutes an absolute proof that there is no such thing as Luxus consumption of meat in the sense of the hypothesis of Frerichs' and of Schmidt's; i. e., that an oxidation of food protein in the blood takes place without previous incorporation with the nitrogen-containing parts of the body tissue.

Sugar reduces the protein metabolism in the organs of the body and reduces the quantity of protein in the food needed for replacement purposes, and possesses these influences even more than fat, probably because it has a greater

affinity for oxygen than either ingested fat or body fat. . . . Starch behaves like sugar.

It is established for all time and is and must be correct that the nitrogen-containing materials are the sources of physical power, of the phenomena of motion; also it is equally incontrovertible that fat and the so-called carbohydrates can yield only heat and never motion. From the foregoing results it follows that the doctrine of Liebig regarding the division of the foodstuffs into *plastic* and *respiratory* is correct.

The authors later suggest the names "dynamogenetic" or "kinetogenectic" for "plastic" food substances, and "thermogenetic" for "respiratory" foodstuffs.

The extension of the work to man is desirable. It should be known to what extent ingested protein nitrogen appears in his urine as urea or whether it is eliminated in other forms.

They expect people to say, "It is all self-evident and we have always known these things," and still others to say, "This is not true, here are facts which contradict you."

It is of great interest to note the affirmation of the doctrine of Liebig in this early work, that though muscle effort was the cause of the metabolism of protein, oxygen caused the destruction of fat and carbohydrate up to the limit of the quantity of oxygen available. Both of these doctrines were subsequently overturned by Voit. In the first place, he found, the very same year as that in which he published his work with Bischoff, that muscular work did not increase the protein metabolism of a fasting dog or of one fed with meat. Later he showed the same to be true in the case of a fasting man and of a man fed with a mixed diet containing a liberal amount of protein. He writes: "I maintain this as an incontestable fact. It is of itself so important that I question whether it is desirable to add a word of explanation. The results of a properly conducted and properly appreciated experiment can never be annuled, whereas a theory can change with the progress of science." How quickly came the upsetting of the former assertion, "It is established for all time and is and must be correct that the nitrogen-containing substances are the sources of physical power, of the phenomena of motion!"

When I was in the Munich laboratory of Voit and happened to make a positive assertion, the then second assistant, Max Cremer, said to me, "Sagen Sie nicht, Herr Lusk, es *ist* so; sagen Sie lieber *möglicherweise es kann so sein.*" Such are the cautious admonitions of those acquainted with history.

The passing of the conception of oxygen being the cause of the metabolism appears from the following words of Voit(*b*), written in 1865: "The conditions of protein metabolism lie in the elementary particles of the organs of the body, which are the hearthstones for all variations and activities. The life of the body is the sum of the action of all the thousands of minute workshops. A combination with oxygen is not first

necessary, but there is a breaking up into various constituents which, under certain circumstances, may remain unoxidized.

"Through the peculiarities of cellular structure the conditions of oxidation are entirely different from those obtaining outside the cells. Under ordinary circumstances nitrogen content means difficulty of decomposition, but in the body, protein is most readily destroyed. Hydrogen is the most inflammable of the gases, but it can be respired up to quantities of hundreds of liters daily without being oxidized.

"What the eye of the layman regards as rest is in reality an interminable movement to and fro of the finest cellular particles, the most complicated of all processes."

Voit's theory of "organized protein" and "circulating protein" served its purpose in emphasizing the difference between the behavior of the living protein of the tissue and the more readily metabolized protein of the ingested food, even though the idea so troubled Liebig that, for the thought of it, he could not tell his right hand from his left, and even though it is now known that protein ingestion does not materially add to the mass of blood protein.

Voit, in his necrology of Pettenkofer (*d*), thus describes a few of the results obtained by their combined efforts with the celebrated respiration apparatus: "Imagine our sensations as the picture of the remarkable processes of the metabolism unrolled before our eyes, and a mass of new facts became known to us! We found that in starvation protein and fat alone were burned, that during work more fat was burned, and that less fat was consumed during rest, especially during sleep; that the carnivorous dog could maintain himself on an exclusive protein diet, and if to such a protein diet fat were added, the fat was almost entirely deposited in the body; that carbohydrates, on the contrary, were burned, no matter how much was given, and that they, like the fat of the food, protected the body from fat loss, although more carbohydrates than fat had to be given to effect this purpose; that the metabolism in the body was not proportional to the combustibility of the substances outside the body, but that protein, which burns with difficulty outside, metabolizes with the greatest ease, then carbohydrates, while fat, which readily burns outside, is the most difficultly combustible in the organism."

In Voit's great textbook, "Der Handbuch der Ernährung und des Stoffwechsels" (1881), one may read the words: "The methods determining the ingo and outgo of metabolic materials for animals and man have very largely been devised by me." It was only Bidder and Schmidt, with a crude respiration device, who had in any way approached the methods of Voit.

It has already been shown how the scientific susceptibilities of nations may be aroused and how two men of different nations may have their disagreements. The polemics which Pflüger, in Bonn, wrote against Voit,

in Munich, have, however, historical interest. Voit (*g*), incensed by the biting criticism of Pflüger, adds a signed postscript to an article by Max Gruber (1881) which concludes as follows: "It is to be regarded as self-understood that I cannot enter into a method of dispute which is so unworthy, a method which I can only despise. In science one should seek to establish the truth by demonstrating the validity of one's opinions after quiet and searching consideration and it is indeed an evil sign when one goes as far as Pflüger has gone in his polemic and uses language which would not be tolerated in good society and would not be regarded as permissible even in excited political debate. Such treatment of scientific problems cannot possibly promote science but only hurt it, and I am sure that many others think as I do, others who through honest endeavor have shown that science was their primary interest, men who have been able to open up new paths therein. It is fortunate that Pflüger, who has no sense of justice, is not the arbiter of the accomplishments of science but rather the future and those contemporaries who can dispassionately estimate the work of others. I declare that I turn away from this hateful discussion with loathing and cannot copy Pflüger in behavior."

To this Pflüger (*i*) answers: "The unvarnished truth of my exactly critical reply has seized Voit so that he was thrown into a paroxysm of raving passion, and setting aside a real answer, he has poured upon me the most insulting invective" (1881).

Answering this in the only purely polemical article he ever wrote, Voit (*c*) replies: "Gruber completely refuted the criticisms of Pflüger concerning our work and clearly explained Pflüger's continual misrepresentation of the same. It only remained for me to rebutt his groundless accusations against the work put out from my laboratory. This could only have been accomplished, not as Pflüger says, in passion and raving, which are foreign to me and hated by me, but rather by quietly explaining in the postscript that I would not reply to remarks of mistrust and calumny, which I can only despise" (1882).

Criticism is invaluable. Pflüger later in life wrote, "Criticism is the mainspring of every advance, therefore I practice it." But the quality of it must not descend to billingsgate. Barker has aptly quoted from "Truthful James,"

> "I hold it is not decent for a scientific gent
> To say another is an ass—at least to all intent;
> Nor should the individual who happens to be meant
> Reply by heaving rocks at him, to any great extent."

Among the problems with which Voit concerned himself was the conversion of starch into fat and of protein into fat and into sugar. His earlier conception was that protein was largely convertible into fat, and

this conception was in his mind to the end. In 1885 it was shown by Rubner in Voit's laboratory that the relation between carbon and nitrogen in meat protein, instead of being 3.68 C : 1 N, was really 3.28 C : 1 N. Seven years after this Pflüger's polemical arraignment of Voit's older work appeared, which was based upon a recalculation of the former experiments of Pettenkofer and Voit (*i*). To this Voit made no reply, since such a recalculation was merely in accord with Voit's later understanding.

At one time I had the good fortune to talk with Pflüger for about half an hour. He saw very few people and the introduction occurred under especially favorable auspices. We discussed the production of sugar from protein, which he freely admitted was possible, though at the time in his writings he was inveighing against the idea. He was cordial, friendly and appeared to me to resemble Voit more closely than any one I had ever seen. His writings seemed to belie the character of the man.

Voit was the first to insist upon the value of flavor in the diet. A food was a *well-tasting* mixture of foodstuffs, he insisted. A food without flavor was rejected by both man and beast.

To give in detail the later historical development appears unnecessary. A Munich review of the German translation of Lusk's "Science of Nutrition" (Stoffwechsel und Ernährung) states that the development of the school of Voit was nowhere else so thoroughly expounded.

Voit was always keenly interested in his lectures and his teaching. He was precise in his statements, clear and interesting. He read his lectures or presented the materials from notes, but no one in the audience could tell whether he was reading from a text, as he often did, or extemporizing. The lecture was in truth a "Vorlesung." He was conscientious in every relation in life. A story is told that when the orders went forth that the university would end on the fifteenth of the month, the professor was greatly disturbed as to whether the order meant "including" or "excluding" the fifteenth. This was at a time when the average professor stopped lecturing when it suited his convenience, and many days before the time set. His own standards which he set for himself were rigid. He was an upright, honest, fearless, kindly man. At one time an assistant, meaning to flatter him, said, "Your views are certainly the right ones," to which he replied in tones of sharp reproof, "It makes no difference *who* is right so long as the truth is ultimately achieved."

Rubner, Erwin Voit (a brother), Friedrich Müller, F. Moritz, Fritz Voit (a son), Straub, Ellinger, Otto Frank, Prausnitz, Gruber, Cremer, Weinland, Heilner, Atwater and I all owe allegience to the Munich school of Voit.

Voit taught that one case carefully investigated was worth more than many hundreds casually examined.

On the practical side, his investigations showed that an average laboring man consumed food containing 118 gm. of protein and about 3,000

calories, or approximately the same diet as had been estimated by Playfair in 1865 (see p. 78). The unit of 3,000 calories was adopted as the requirement of energy for the average adult male citizen when the Inter-allied Scientific Food Commission met in Paris at the end of March, 1918, to determine standards for the provisioning of a population of 225,000,000 people. The battleground around the 118 gm. of protein has been active for forty years, with no greater result than the well-defined impression that those who take that quantity of protein have a greater virility than those to whom it is denied.

In the laboratory Voit was always enthusiastic. A new discovery was the cause of joy. The figures to be obtained excited his curiosity, he would say, or the results were most interesting, most important. The new method was extraordinarily accurate and the expectations therefrom fascinating.

One day I burned my hand with ether in the laboratory. Some one went for some cocain to relieve the pain, for which I offered to pay. Money was refused. I had done so much for the State that the State could well afford to pay. It was a new conception to me of a fundamental relation of experimental laboratory work to the welfare of the State.

I look back upon my days in Munich with gratitude and to the memory of Voit with respect and veneration.

Of those who were educated in the atmosphere of the Munich school of Voit, Friedrich von Müller is preëminent among physicians as the leading internist of his time. And Rubner was the first to solve the problem initiated by Lavoisier, of demonstrating that the law of the conservation of energy held true for the animal organism.

Max Rubner (1854-....).—While still in Voit's laboratory as first assistant Rubner (*d*) determined the calorific value of urine and feces under different dietary conditions and laid the foundations for the computations involved in modern animal calorimetry (1885). Rubner applied the knowledge he had won to the calculation of the heat production in man and in many animals of different species. He (*e*) evolved the law of surface area, that the heat value of the metabolism of the resting individual is proportional to the area of the body surface. This law had been previously indicated in the writings of Regnault and Reiset, as has been shown (p. 43). His first publication regarding this was in 1883. A good review of the literature on this subject is given by Benedict (*z* 1919).

Voit had constructed a calorimeter for measuring the heat production of man and extensive and laborious experiments were carried out with it during the years 1869, '70, '71, '74 and 1884. The mass of material was never published on account of the imperfection of the apparatus.

Rubner (*e*), in 1891, working in his own laboratory at Marburg, virtually with his own hands and with a very small allowance of money, made a calorimeter which accurately measured the heat production of an animal.

The interior of the apparatus was connected with a Pettenkofer-Voit respiration apparatus. The heat measured by *direct calorimetry* agreed within a fraction of one per cent with the heat calculated from the metabolism products by *indirect calorimetry*. Voit, when he heard of this triumph of technic, remarked that it was the greatest discovery in its way since the invention of the thermometer.

Fig. 8. Max Rubner. From a photograph taken in New York in 1912.

Rubner's insistence upon the importance of the energy relations was especially upheld in his volume, "Die Gesetze des Energieverbrauchs bei der Ernährung," published in 1902. On account of the difficulty of the style of presentation adopted in this book it was some time before its suggestiveness was appreciated. Entirely different in style and finely written in his more popular "Kraft und Stoff in Haushalt der Natur," published in 1909.

Rubner is a man who finds his relaxation among artists and can himself paint a picture; a man of great talents and fine personality. It is interesting to note that his advice on the food problems was largely disregarded by the German authorities during the war (1914-18), and that his prophecies regarding what would happen were fulfilled.

Nathan Zuntz (1847-1920).—No history of metabolism would be complete without mention of Zuntz, in his early days a pupil and assistant of Pflüger, a practitioner of medicine for ten years, and long chief of the agricultural college in Berlin. Zuntz studied the metabolism by means of the gas analysis of the expired air obtained in short periods, and devised a portable apparatus for the measurement of the metabolism of a man walking at the sea level or on the snow fields of Monte Rosa. He made

several balloon ascensions for scientific purposes. He also measured the cost of energy at which horses and cattle performed work, and the loss of energy through the bacterial putrefaction of the foods in such herbivora. Magnus-Levy, a pupil, carried the Zuntz respiration apparatus to the bedside of hospital patients and made pioneer investigations the validity of which has been generally confirmed. Zuntz had a quiet, attractive personality, without, however, possessing the breadth of view of Rubner, who was the most frequent antagonist of his views.

Late French Work

If we turn back to France for a moment, which we left in the year 1849, we find an important paper by Berthelot (1827-1907) entitled "Sur la chaleur animale," published in 1865, in which he argues concerning the differences in the quantities of heat produced when equal weights of carbohydrate and fat are oxidized in the body. He points out that it is impossible to determine the heat production in the body by means of the method of Lavoisier because 44 gm. of carbon dioxid produced from the oxidation of carbon yield 94 calories, whereas the same amount produced from methane yields 210,000 calories. He thus early concludes that 'the quantity of heat liberated in the incomplete oxidation of a substance is equal to the difference between the total caloric value of the substance and that of the products formed."

Rubner's calorimetric observations were the realization of this theoretical conception.

The experiments of Charles Richet (1850-. .), published in 1885, confirmed Rubner's Law of Surface Area, and Richet affirms that in future one should express all calorimetric observations in terms of surface area and not in weight, a principle now being largely followed in the United States. Richet compared the heat production, as measured by his calorimeter, of a cat, rabbit and goose of equal weights, as follows:

	Weight in grams	Calories per kilogram per hour
Cat	3135	3.30
Rabbit	3100	3.32
Goose	3310	3.32

Writing about this work, in 1889, he says: "Let us consider a horse, for example, which weighs 525 kg. and having a radius, one may assume, of 50 centimeters, the surface area would then be 31.5 square meters. This area is the same as that of 2250 sparrows, each weighing 20 grams. Consequently, sparrows weighing 45 kilograms have the same surface as a horse weighing 525 kilograms."

In the summer of 1920, in Paris, Richet, in his opening address as president of the Physiological Congress, said, "Seek to understand things; their utility will appear later. First of all it is knowledge which matters." And he illustrated this by citing the investigations of Claude Bernard on the glycogenic function of the liver and the investigations of Portier and himself, while they were sailing through tropical waters on the yacht of Prince Albert of Monaco, upon the subject of anaphylaxis which they carried on with poisons of sea anemones injected into birds.

Conclusion

The writer is conscious of the fact that this story is incomplete. For example, he is not forgetful of the work of Lyon Playfair (1818-1898), a pupil of Ludwig who in 1865 gave various dietary standards among which that for a man working moderately was about the same standard fixed later by Voit. Nor does he forget the recent work of Robert Tigerstedt of Helsingfors, or of Tangl (1866-1916) of Budapest, of Johannson of Stockholm. The complete story would be long, too crowded with details, perhaps already a justifiable criticism of the material here presented.

In a recent address given in Berlin, Friedrich Müller stated that the science of nutrition, which had been a German science, had partly passed to America. But before it became German it was French, perhaps before that English, and at its dawn Italian. In this country the early calorimetric work of Wood and Reichert, both of Philadelphia, ought not to be forgotten. Wood's work on fever is of importance. The work of Chittenden (a pupil of Kühne of Heidelberg), continued by Mendel; of Atwater, continued by Armsby, F. G. Benedict and H. C. Sherman; that of McCollum, a pupil of Mendel; of Steenbock, a pupil of McCollum; that of Murlin, Du Bois, Ringer and me, has been work accomplished in the earnest endeavor to unfold the truth as we have understood our mission. We are not unmindful of the aid given by those of more purely chemical tastes, like Osborne, Folin, Levene, Stanley Benedict, Jones, Van Slyke, and Dakin; or of the mighty travail of Alonzo Taylor, chief scientific adviser to Herbert Hoover in his work of providing nourishment for the nations of the world.

Across the water in that wonderful island called Great Britain are Hopkins, T. B. Wood, Halliburton, Cathcart, Leonard Hill, Hardy, E. H. Starling and others through whose unrecognized efforts the food program of their country was saved from disaster during the war. Strong scientific personalities have developed in Britain, despite lack of national recognition. These men and men in France, in Italy, as well as in Germany, are carrying on to-day what will to-morrow be a part of the History of Metabolism.

SECTION I

Dietary Constituents and Their Derivatives

The Proteins and Their Metabolism ..... *A. I. Ringer*

Introduction—Elementary Composition of Proteins—Classification of the Proteins—The Structure of the Protein Molecule—Amino Acids or "Building Stones of Protein"—The Rôle of Amino Acids in the Structure of the Protein Molecule—The Amino Acid Content of Different Proteins—Reactions of Protein—Color Reaction—The Biuret Reaction—The Xantho Proteic Reaction—The Million's Reaction—The Sulphur-lead Reaction—The Molisch Reaction—The Adamkiewicz-Hopkins-Cole Reaction—The Triketohydrinden Hydrat Reaction—Precipitating Reactions of Proteins—The "Salting Out" of Proteins by Means of Electrolytes—Coagulation and Denaturalization of Proteins—The Salt Formation of Proteins—The Digestion of the Protein—The Absorption of Products of Protein Digestion from the Gastro-intestinal Canal—The Fate of Absorbed Amino Acids in the Blood—The Fate of Amino Acids in the Tissues—Urea Formation—The Fate of the Non-nitrogenous Fraction of the Amino Acids—Protein Metabolism—The Question of Optimum Versus Minimum Protein Diet—The Function of Protein in the Diet—Incomplete Proteins—The Influence of Protein on Metabolism—The Specific Dynamic Action of Protein.

The Proteins and Their Metabolism

A. I. RINGER

NEW YORK

Introduction

The proteins are the most important constituents of the animal and plant kingdoms. They are an ill-defined group, colloidal in character, non-volatile and obtainable in a pure state with the greatest of difficulty.

Just as the molecules of the simple chemical compounds are built up of atoms and radicals, the protein molecule is composed of the union of a great many amino acids. In all, about twenty-one different amino acids have been found, and there is every reason to believe that more will be found in the course of time. When one realizes that the amino acids themselves are of rather large size and that all of them may be present in most of the proteins, one can readily appreciate the enormous size and complexity of the protein molecule. The exact determination of the molecular weight of the protein seems at present to be a hopeless task, in spite of many ingenious attempts. By means of the freezing point method, egg albumin is found approximately to possess a molecular weight of about 14-000, and calculating the molecular weight of hemoglobin on the basis of one atom of iron, one gets the figure of 16000. The protamins, which are the simplest proteins, have a molecular weight of approximately 4000.

Elementary Composition of Proteins

The proteins are composed of the following elementary constituents: Carbon, Hydrogen, Nitrogen, Oxygen and Sulphur. The quantitative relationship of these elementary constituents is found to fluctuate in the different proteins within narrow limits. Carbon, 50 to 55 per cent; hydrogen, 6.5 to 7.5 per cent; nitrogen, 15 to 17.5 per cent; sulphur, 0.3 to 2 per cent; phosphorus, 0.4 to 0.8 per cent; oxygen, 21 to 23 per cent.

Classification of the Proteins

Up to the present we have not yet arrived at any definite knowledge concerning the structural formula of the protein molecule, and until that

is achieved a satisfactory chemical classification will not be possible. All the known proteins possess certain chemical and physical properties in common, and differ in others. The classification at present is based on these differences. It is based upon differences in their solubilities, coagulations, precipitations, etc. It is a crude, and more strictly physical than chemical classification, but it answers the purpose to a certain extent by bringing some order out of chaos.

The proteins are divided into three main groups:

I. *The simple proteins* which yield on hydrolysis α-amino acids.

II. *Conjugated proteins* which are composed of simple proteins chemically united with another organic compound.

III. *Derived proteins* which are proteins that are found in the incomplete digestion or hydrolysis of either of the above naturally occurring protein.

These three main groups may be further subdivided into the following groups:

I. Simple Proteins.

a. Albumins
b. Globulins
c. Glutelins
d. Prolamins (alcohol soluble proteins)
e. Albuminoids or Scleroproteins
f. Histones
g. Protamins

II. Conjugated Proteins.

a. Nucleoproteins
b. Glucoproteins
c. Phosphoproteins
d. Chromoproteins
e. Lentoproteins

III. Derived Proteins.

A. Primary
a. Proteins
b. Metaproteins
c. Coagulated proteins

B. Secondary
a. Proteoses
b. Peptones
c. Peptides

The *albumins* are present extensively in the animal and plant kingdoms. The most important ones of this group are serumalbumin (from blood), ovalbumin (from the white of egg), lactalbumin (from milk).

As a class they are characterized by their solubility in distilled water, dilute acid and alkali. In the presence of neutral salts they are coagulated

by heat, and are precipitated by alcohol, concentrated mineral acids and the salts of heavy metals. They are quantitatively precipitated by saturation with ammonium sulphate in neutral solution. Most of them may be obtained in pure crystalline form.

The *globulins* are also present extensively in the animal and plant kingdoms. They are found in the blood as serum globulin, fibrinogen and its derivative fibrin; in the muscles as myosinogen and myosin; in the egg as ovoglobulin; in milk as lactoglobulin; in the crystalline lens of the eye as lentoglobulin; in the thyroid gland in combination with iodin as thyreoglobulin or iodothyreoglobulin; in the urine as Bence Jones' protein.

As a class they are characterized by their insolubility in pure distilled water and dilute acid solutions. They are, however, soluble in dilute neutral salt solutions and in dilute alkaline solutions. They are coagulated by heat and precipitated by alcohol. They are completely precipitated by saturation with magnesium sulphate and by half saturation with ammonium sulphate. They are strongly acid in reaction and possess the power of turning blue litmus red.

The *glutelins* are a group of proteins which are present in the plant kingdom only. We know the glutelin of wheat and the oxyzenin of rice. They are soluble in dilute alkali, forming salts.

The *prolamins* or *alcohol soluble proteins* are a group of proteins found in cereals. They are gliadin of wheat, hordenin of barley and zein of maize. They are characterized by their solubility in 70 to 80 per cent alcohol, and by their insolubility in water, neutral solvents and absolute alcohol.

The *albuminoids* or *scleroproteins* are a group of proteins found in the framework of all connective tissues. In this group belong elastin, gelatin and collagen, keratin from hair, bones, hoofs, nails, turtle shell, also silk gelatin, reticulin, etc. They are characterized by their marked insolubility in any of the neutral solvents and their resistance to chemical decomposition.

The *histones* are a sharply defined group of proteins strongly alkaline in reaction, and not found free in nature but in combination with acids or other proteins. They contain a large amount of the dibasic amino acids (see page 87), lysin, arginin and histidin. They are found in combination with nucleic acid in the nucleoproteins and with hematin in hemoglobin. They are soluble in water and precipitated by alkali. They are coagulated by heat in the presence of small amounts of salts, and are precipitated by other proteins.

The *protamins* are the simplest of all the proteins. Similar to the histones, they are strongly alkaline in reaction. They contain 25 to 30 per cent of nitrogen and are made up almost entirely of the dibasic amino acids (ninety per cent). They are found in combination with nucleic acid in the nuclei of the spermatozoa of numerous fish. They are soluble

in water, and are not coagulated by heat. They turn red litmus blue. Because of their basicity they have the power of absorbing carbon dioxid from the air, forming carbonates. They form stable salts with mineral acids and have the power of precipitating other proteins.

The *conjugated proteins* will be taken up in a separate chapter. The *derived proteins* will be discussed in the chapter on digestion.

The Structure of the Protein Molecule

It has been known for a long time that if acids, alkalis or digestive ferments like pepsin or trypsin be allowed to act on protein under suitable conditions, there sets in a decomposition of the protein molecule, which, if carried on for a long enough time, will cause an almost complete disappearance of the protein. In the process of this decomposition a number of cleavage products are produced which have been isolated, purified and identified. They are all amino acids—i. e., organic acids which have an amino ($—NH_2$) radical attached to their α-carbons. These amino acids are obtained from the splitting of all proteins, and because of that they are known as the "building stones" of protein. To date, twenty-one different amino acids have been obtained as cleavage products of the protein molecule, and there is every reason to believe that the list is not yet complete, though it may be said with certainty that the most important ones have been accounted for.

Amino Acids or "Building Stones of Protein"

The known amino acids may be considered under the following headings:

A. Monobasic Mono Amino Acids.

1. Glycocoll or glycin or α-amino acetic acid.

$$\begin{array}{l} CH_2NH_2 \\ | \\ COOH \end{array}$$

2. Alanin or α-amino propionic acid.

$$\begin{array}{l} CH_3 \\ | \\ \alpha\text{-}CHNH_2 \\ | \\ COOH \end{array}$$

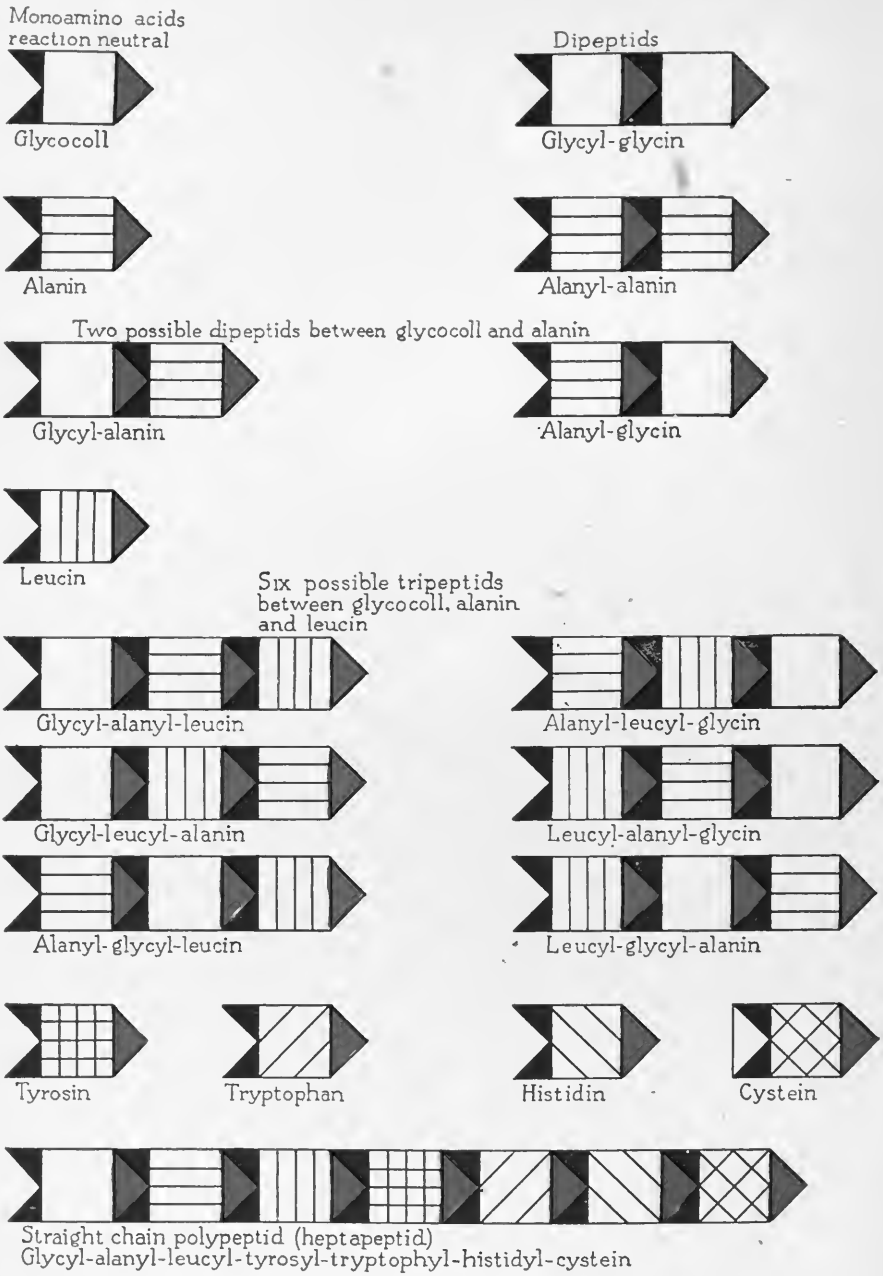
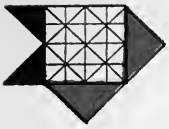


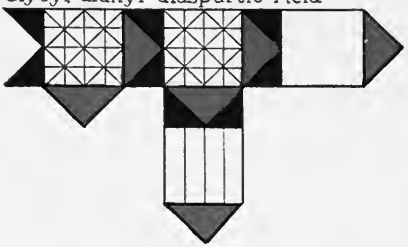
PLATE I. SCHEMATIC REPRESENTATION OF THE AMINO ACIDS.

The neutral amino acids each contain a basic amino group (blue) and an acid carboxyl group (red), which neutralize each other. These amino acids can link themselves to one another in straight chains, in any combination and permutation, the amino group uniting with the carboxyl group.



Aspartic Acid.
Dibasic acid,
reaction acid

Branched Polypeptid.
Glycyl-alanyl-diaspartic-Acid



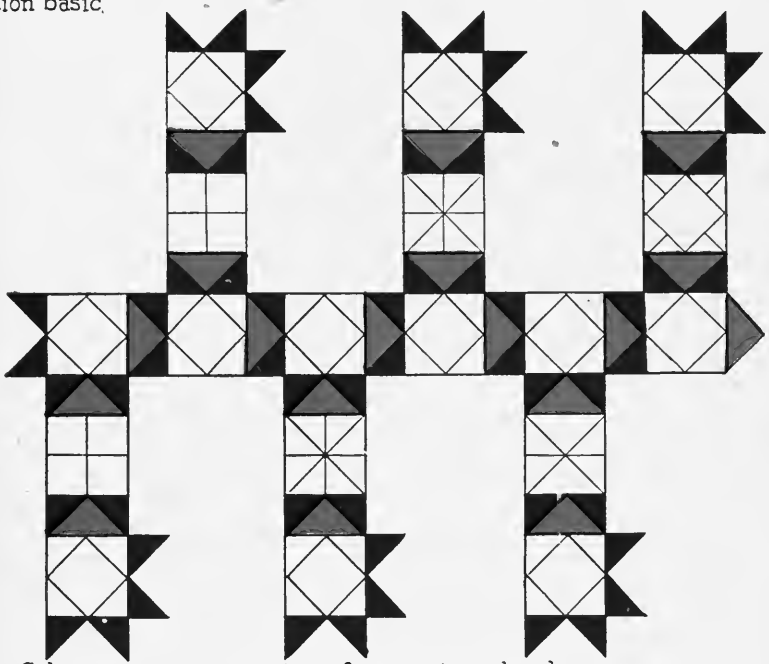
This tetrapeptid can develop linkages along two branches, beside the main chain. Its reaction is acid due to the preponderance of carboxyl groups. Such polypeptids linked would give an acid protein.

Arginin
Diamino acid,
reaction basic

Serin

Prolin

Valin

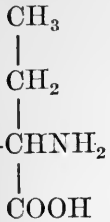


Schematic representation of a protein molecule

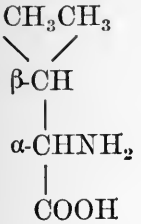
PLATE II. SCHEMATIC REPRESENTATION OF A PROTEIN MOLECULE.

This is the supposed composition of the protamin of the salmon (salmine). Six tripeptids, each composed of two molecules of the diamino acid arginin and one monoamino acid, are linked together. The protein is strongly basic because of the preponderance of the amino groups.

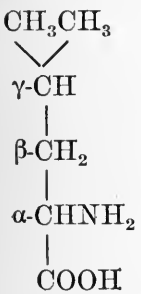
3. α-amino butyric acid.



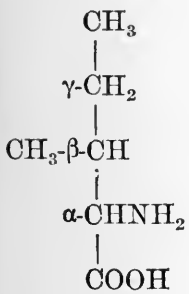
4. Valin or α-amino iso-valerianic acid or α-amino β-methyl butyric acid.



5. Leucin or α-amino γ-methyl valerianic acid.



6. Iso-leucin or α-amino β-methyl valerianic acid.



7. Normal Leucin or α-amino caproic acid.

$$\begin{array}{l} CH_3 \\ | \\ \delta\text{-}CH_2 \\ | \\ \gamma\text{-}CH_2 \\ | \\ \beta\text{-}CH_2 \\ | \\ \alpha\text{-}CHNH_2 \\ | \\ COOH \end{array}$$

These amino acids are neutral in reaction, but have the property of uniting with both acids and alkali. Glycocoll, for example, can combine with NaOH forming sodium glycocollate:

$$\begin{array}{lcccl} CH_2NH_2 & & & & CH_2NH_2 \\ | & + & NaOH & \longrightarrow & | \quad + \ H_2O \\ COOH & & & & COONa \\ \text{Glycocoll} & & \text{Sodium hydroxid} & & \text{Sodium Glycocollate} \end{array}$$

which is still capable of combining with an acid radical, because of the free basic amino radical ($—NH_2$).

On the other hand, glycocoll can combine with an acid like hydrochloric, forming a well defined salt, glycocoll hydrochlorid, which is acid in reaction.

$$\begin{array}{lcccl} CH_2NH_2 & & & & CH_2NH_3Cl \\ | & + & HCl & \rightarrow & | \\ COOH & & & & COOH \\ \text{Glycocoll} & & \text{Hydrochloric acid} & & \text{Glycocoll hydrochlorid} \end{array}$$

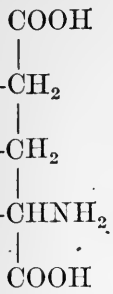
and is capable of uniting with alkali because of its free acid radical ($—$ COOH) known as carboxyl.

B. Dibasic Mono Amino Acids.

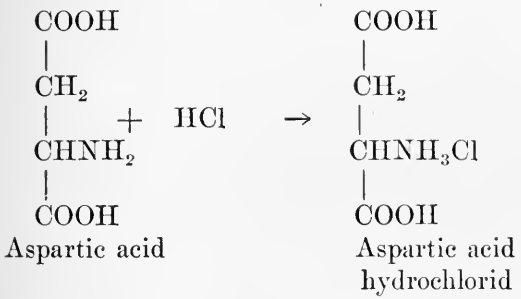
1. Aspartic acid or α-amino succinic acid.

$$\begin{array}{l} COOH \\ | \\ \beta\text{-}CH_2 \\ | \\ \alpha\text{-}CHNH_2 \\ | \\ COOH \end{array}$$

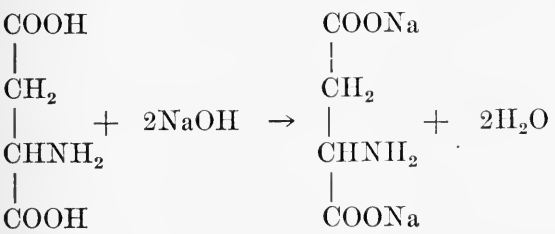
2. Glutamic acid or α-amino glutaric acid.



These amino acids are strongly acid in reaction because of the fact that they possess two acid radicals and only one base. In spite of the fact that they are strongly acid, they possess the power of combining with other acids, forming salts.

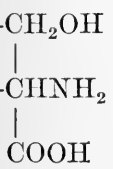


They also have the power of combining with two alkali radicals because of the two carboxyl (— COOH) radicals.

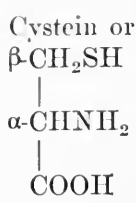


C. Hydroxy- and Thio-α-amino acids.

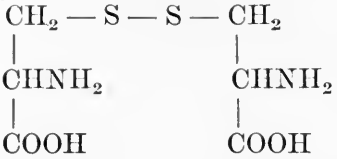
1. Serin or α-amino β-hydroxypropionic acid.



2. Cystein or α-amino β-thio-propionic acid.

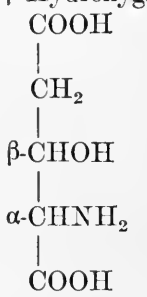


3. Cystin or dicystein.



These three substances are neutral in reaction, and have properties similar to those in group "A". The two latter are the only amino acids which contain sulphur, and there is every indication to prove that only the latter exists in protein and that the former is only a product of its hydrolysis.

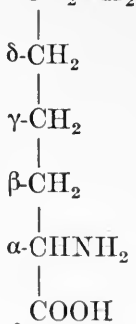
4. β-Hydroxyglutamic acid, Dakin (1918, 1919).



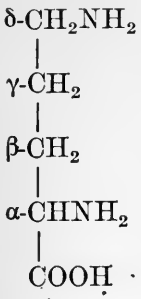
This acid is similar to the dibasic acid glutamic acid, except that it has an hydroxyl radical attached to the β-carbon. This is the youngest member of the amino acid family, having been discovered by H. D. Dakin in 1918.

D. Diamino acids.

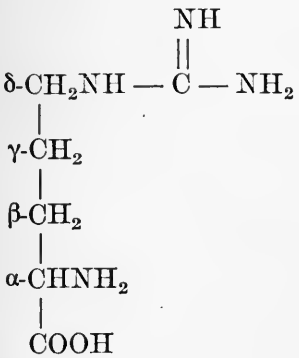
1. Lysin or α-ε-diamino caproic acid.



2. Ornithin or α-amino δ-amino valerianic acid.



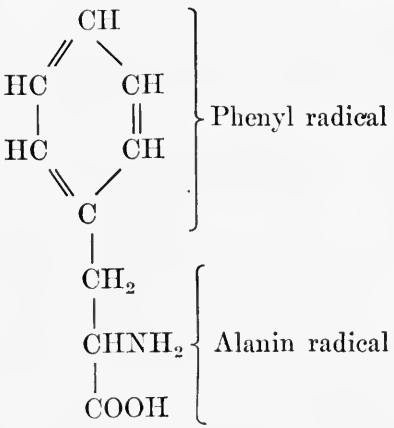
3. Arginin or α-amino δ-guanidin valerianic acid.



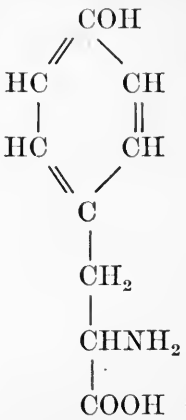
These substances are strongly alkaline in reaction. The last substance, on hydrolysis with alkali or an enzyme known as arginase, splits into urea and ornithin. This latter substance is not found as such among the protein cleavage products.

E. Aromatic amino acids.

1. Phenyl-alanin or α-amino β-phenyl propionic acid.



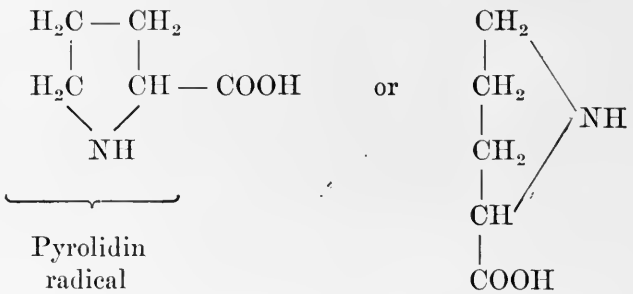
2. Tyrosin or α-amino para hydroxy phenyl propionic acid.



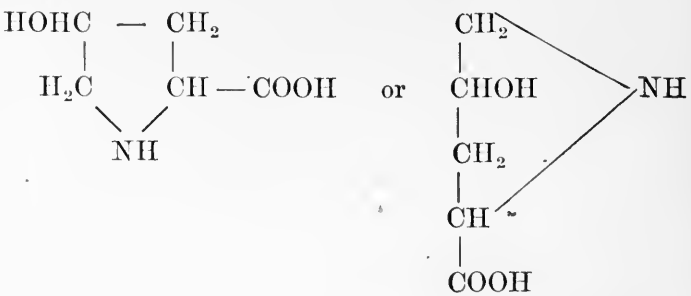
These amino acids are similar to those of the monobasic mono-amino acid group, except that they are derivatives of the phenyl group.

F. Heterocyclic amino acids.

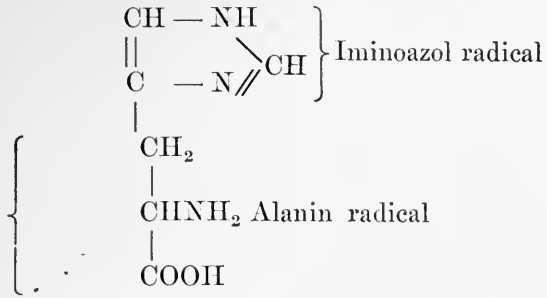
1. Prolin or α-pyrolidin carboxylic acid.



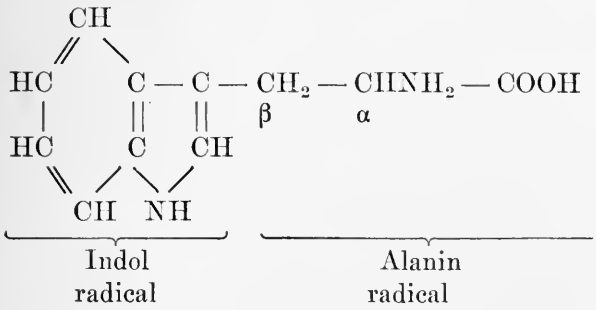
2. Oxyprolin or hydroxypyrolidin carboxylic acid.



3. Histidin or α-amino β-*iminoazol propionic acid.*



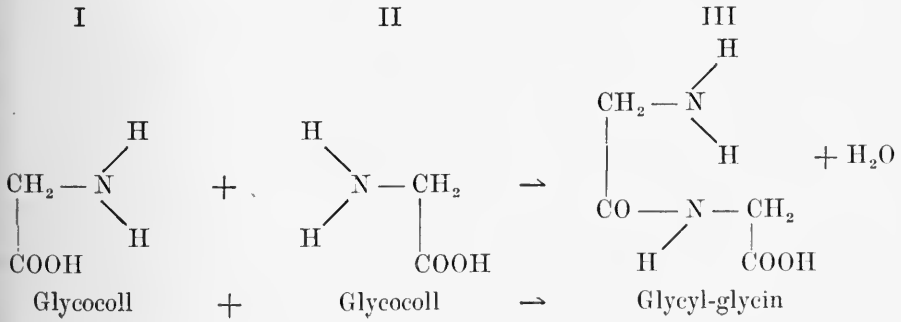
4. Tryptophan or Indol α-amino propionic acid.



The Role of Amino Acids in the Structure of the Protein Molecule

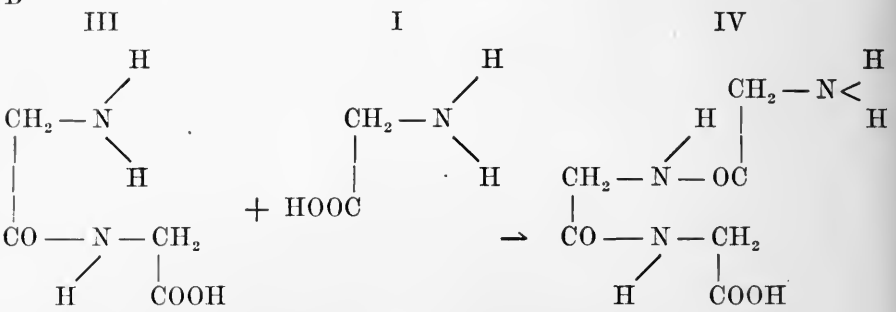
From the above it is seen that all the amino acids, no matter how simple or complex their structure, possess at least one amino (— NH_2) radical and at least one acid (— COOH) radical. These two radicals impart to each amino acid the power of uniting with at least two other amino acids of similar or different structure, forming what are known as peptids.

A

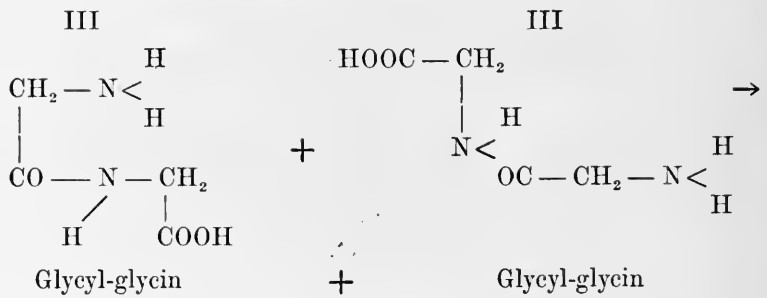


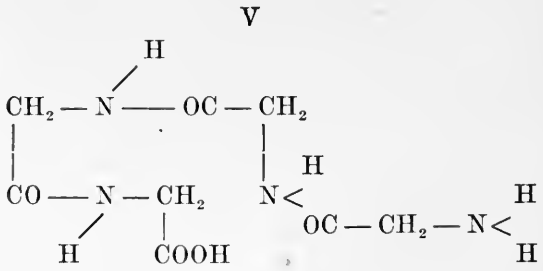
In this reaction two glycocoll molecules are allowed to interact. The basic amino radical of II unites with the acid carboxyl radical of I, giving rise to the glycyl-glycin peptid III. This compound, while larger and more complex than the original glycocoll, still possesses one free $—NH_2$ and one free —COOH at either end, which again makes it capable of uniting with other amino acids at either end or with other peptids.

B



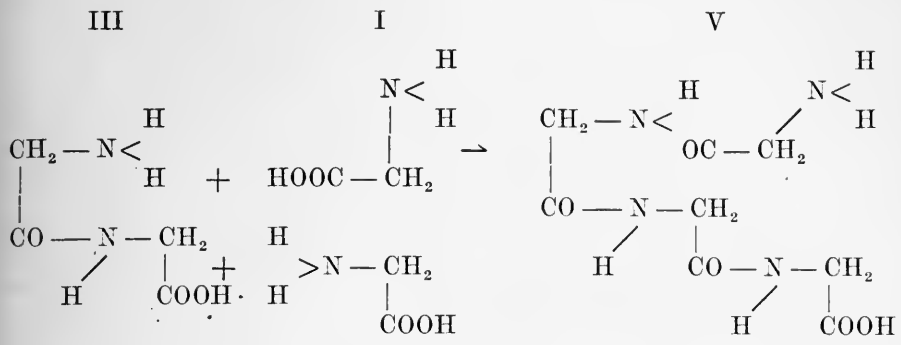
C





Tetra-glycyl-glycin

D



Glycyl-glycin + 2 molecules of glycocoll → Tetra-glycyl-glycin

In these reactions we have illustrations of the various reactions that glycocoll and its peptids may undergo. In B. we have a molecule of glycyl-glycin unite with one molecule of glycocoll, giving rise to a tri-peptid glycyl-glycyl-glycin.

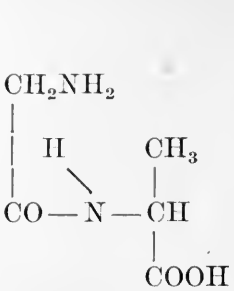
In C. one molecule of glycyl-glycin unites with another molecule of glycyl-glycin, giving rise to a tetra-peptid, while in D. one molecule of glycyl-glycin unites with two molecules of glycocoll, giving rise to the same tetra-peptid.

From these illustrations we also learn that no matter how many amino acids are hooked on to one another, they will always have one $—NH_2$ free at one end, and one —COOH at the other, making the possibility of the length of this chain indefinite.

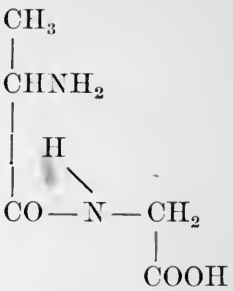
We may therefore conceive of an amino acid as an individual with an arm at either side, capable of clasping two other individuals. The chain that may thus be formed is theoretically endless.

If a protein were made up by the union of a large number of molecules of a single amino acid the problem would be comparatively simple. We would be dealing with a straight chain of amino acids. The difference between one protein and another would depend only upon the number of amino acid molecules that go to make the protein molecule. But in the natural proteins we have to deal with a union of about twenty-one amino acids, which introduces an entirely new factor, namely that of isomerism and stereo-isomerism.

Only one kind of union is possible between glycocoll and glycocoll. Between glycocoll and alanin, however, two unions are possible, glycyl alanin and alanyl-glycin.



Glycyl-alanin



Alanyl-glycin

That there is a difference between these two compounds we know from the fact that they behave differently in their physical property of rotating the plane of polarized light. Glycyl-alanin rotates the plane of polarized light 50° to the left, whereas alanyl-glycin rotates it 50° to the right (Abderhalden and Fodor, 1912).

In the union of glycocoll, alanin and leucin, we have six different possible combinations, depending upon the position each amino acid occupies in the molecule with reference to the other amino acids. That there is a difference between these compounds we know from the fact that they all have a different power of rotating the plane of polarized light: Thus:

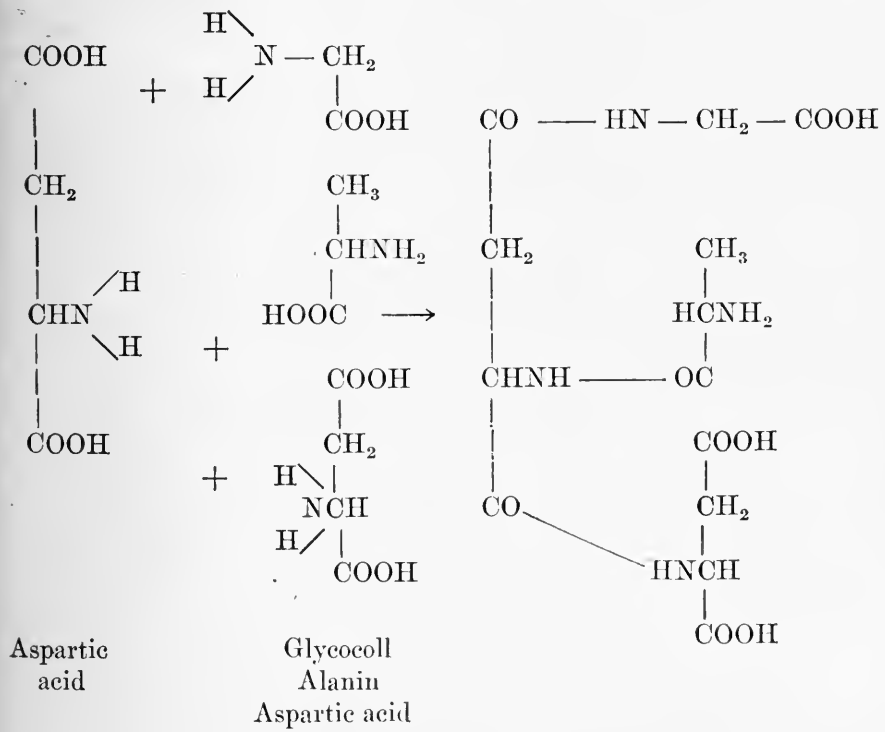
I.	Glycyl-alanyl-leucin	$[\alpha]^{20}_{D} = -90°$
II.	Glycyl-leucyl-alanin	$[\alpha]^{20}_{D} = -60°$
III.	Alanyl-glycyl-leucin	" $= -11°$
IV.	Alanyl-leucyl-glycin	" $= -30°$
V.	Leucyl-alanyl-glycin	" $= -17°$
VI.	Leucyl-glycyl-alanin	" $= +20°$

With the increase in the number of amino acids the number of isomers increases tremendously, as the following table taken from Abderhalden shows:

Number of amino acids	Number of possible compounds
2	2
3	6
4	24
5	120
6	720
7	5,040
8	40,320
9	362,880

Number of amino acids	Number of possible compounds
10	3,628,800
11	39,916,800
12	479,001,600
13	6,227,020,800
14	87,178,291,200
15	1,307,674,368,000
16	20,922,789,888,000
17	355,687,428,096,000
18	6,402,373,705,728,000
19	121,645,100,408,832,000
20	2,432,902,008,176,640,000

If it were possible to arrange twenty amino acids in one straight line forming a protein molecule, 2,432,902,008,176,640,000 different kinds of protein molecules could be formed. This figure, however, does not yet by any means complete the list. While most of the amino acids are able to form unions with other amino acids in a straight line, the dibasic mono-amino acids and the diamino acids are able to form branched chain compounds.



In this reaction we see the possibility of a molecule of aspartic acid uniting with one molecule of glycocoll, one of alanin, and one of aspartic acid; the resultant tetra-peptid has one free $-NH_2$ and three $-COOH$ radicals, which means it can further form compounds along two branch lines outside of the original line. The different possibilities can be best illustrated graphically.

From the above consideration one can readily see the difficulties that confront the investigator of the chemistry of the proteins, and when one also realizes that one cannot claim to understand the nature of a chemical compound until he has a knowledge of its structural formula, one can readily appreciate how far from our goal we are. One can then also realize how crude is our classification of proteins that has been given above. Under the heading of what we call *albumins* we may have billions of different proteins, resembling one another in some respects, and differing in others.

The Amino Acid Content of Different Proteins

Until the technique of the quantitative determination of the amino acids reaches the point where it will be possible to recover 100 per cent of amino acids from a known mixture, an exact answer to the problem of the amino acid content cannot be given. The figures we can gather to-day are therefore more of relative value than of absolute.

Not all proteins contain all the amino acids. We shall learn later that from the nutritional point of view proteins are divided into "complete" and "incomplete" and that under the latter we include those proteins which lack some of the amino acids which are essential for the maintenance of proper nutritional conditions of animals, like tryptophan, tyrosin, lysin or cystin.

Reactions of Proteins

Color Reaction.—The proteins give a number of color and precipitating reactions, which are characteristic of a group, though not specific.

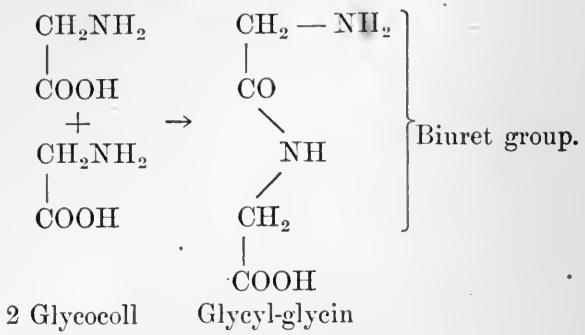
The Millon's Reaction.—When a protein is boiled in Millon's reagent, which consists of a mercury solution in nitric acid and to which a small amount of nitrous acid is added, the solution will turn rose colored to dark red. This reaction is given by all substances having an oxyphenyl radical. In the proteins it is the tyrosin radical which gives this reaction. Proteins, like gelatin, which do not contain tyrosin, do not give this reaction.

TABLE I

RELATIVE AMINO ACID CONTENT OF DIFFERENT PROTEINS

Proteins	Lysin	Arginin	Valin	Oxyprolin	Prolin	Leucin	Glutamic Acid	Aspartic Acid	Tryptophane	Histidin	Cystin	Serin	Tyrosin	Phenylalanin	Alanin	Glycocoll
Serum albumin	+	+		+	1	20	8	3	+		2.3	1	2	3	3	0
Egg albumin	2	2		+	2	8	8	2	+		0.2	+	1	4	8	0
Lac-albumin	+	+	+	1	4	19	10	1	+	+	+	+	1	2	53	0
Cotton-seed edestin	1	11	+	2	2	20	6	4	+	1		1	2	2	4	4
Serum globulin		+	+		3	18	8	2	+		1		2	54	2	3.5
Sunflower edestin			+		3	13	13	3	+			1	2	4	4	2.5
Globulin from toga-bean	3	5	1		4	9	19	4	+	1.4			2	4		1
Legumin	5	5	1		6	9	16	4		1			3	2	3	1
Gliadin	0	3	+		2	6	36	1	1	2	+	+	2	2	53	1
Zein	0	1	+		6	18	18	1	0	+		+	3	5	2	0
Hordein	0	2			9	6	33	+	+	+		+	1	3	1	+
Glutin	2	5	+		4	6	23	+	+	2	+	+	4	2	5	+
Leucosin	3	6	+		3	11	6	3	+	3			3	4	4	+
Avenin			2		5	15	18	4					1	3	2	1
Fibrin	4	3	1		4	15	10	2	+	+	1	1	3	3	4	3
Casein, cow	6	5	1	+	3	10	11	1	1.5	3	+	+	5	3	1	0
Thymus, histon	7	15			1	12	+		2				5	2	3	+
Globin, hemoglobin	4	5		1	2	29	2	4	+	11	0.3	1	2	4	4	0
Protamin, salmon	0	87	2	0	7		0	0	0	0	0	5	0	0	0	0
Strurin	12	60	0	0	0				0	13	0	0	0	0	+	0
Scombin	0	89	0	0	4		0	0	0	0	0	0	0	0	7	0
Silk fibroin	+	1	0		+		0	+		+		2	10	1	2.3	3.6
Elastin		+	1		2	21	+	+					+	4	6	2.6
Spongin		+			7		18	5			7	+		0	0	14
Keratin	0	3	5		4	18	3	3					5	3	1	+
Wool keratin					4	12	12	2			8	+		0	4	1
Gelatin	3	8	1	3	5	2	1	+	0	+	2	2	0	0.5	1	16

The Biuret Reaction.—When protein is treated with a strong sodium hydroxid solution and then a few drops of a very dilute copper sulphate solution is added, a beautiful violet blue color develops. This reaction is due to the presence of the Biuret group.



2 Glycocoll Glycyl-glycin

All proteins and polypeptids give this reaction.

The Xanthoproteic Reaction.—When a protein is boiled with strong nitric acid, a yellow solution is formed, which after making alkaline with sodium hydroxid, turns red brown, and with ammonia, turns orange red. This reaction is due to the presence of the benzene group.

The Sulphur-lead Reaction.—When protein is heated in a solution of sodium hydroxid in the presence of lead acetate, a black color is produced, due to the presence of sulphur in the protein molecule. This reaction in protein is produced by cystin.

The Molisch Reaction.—When a few drops of an alcoholic solution of α-napthol is added to a protein solution and this mixture stratified upon concentrated sulphuric acid, a beautiful violet mixture is formed at the point of contact. This reaction is not given by the proteins themselves but by the carbohydrate radical which is frequently bound to certain proteins (glucoproteins).

The Adamkiewicz-Hopkins-Cole Reaction is obtained when to a solution of protein a small amount of glyoxylic acid is added, and the mixture stratified upon concentrated sulphuric acid. A beautiful violet blue color develops at the point of contact. This reaction is given by the amino acid tryptophan, and proteins which do not contain this amino acid, like gelatin, zein, protamins, etc., do not give it. The presence of sodium or potassium nitrate will interfere with this reaction.

The Triketohydrinden Hydrat Reaction (*Ninhydrin*).—When a small amount of 0.1 per cent of triketohydrinden hydrate is added to a dilute protein solution, and the mixture boiled for a minute or two and then allowed to cool, a beautiful blue color will develop. This is characteristic

of all proteins and is given due to the presence of an α-amino radical next to a free carboxyl (—COOH).

Precipitating Reactions of Proteins.—All proteins are precipitated by absolute alcohol. With dilute alcohol the precipitating point of the different proteins is different, and C. Tebb, 1904, has worked out a means of differentiating between different proteins.

Various mineral acids, like nitric, metaphosphoric, and ferrocyanic acid, as well as the alkaloidal reagents like phosphotungstic, phosphomolybdic, tannic and picric acids, potassium mercuric iodids and potassium bismuth iodids, have the power of precipitating the proteins.

Practically all the salts of the heavy metals have the power of precipitating the proteins. Those that are employed for that purpose most frequently are ferric chlorid, ferric acetate, copper sulphate, mercuric chlorid, basic or neutral lead acetate, zinc acetate and uranyl acetate. The strongly basic proteins, like histones and protamins, also possess the power of precipitating the proteins. Most of the above precipitations are irreversible, i. e., by removing the precipitating agent the proteins cannot be dissolved in water. On adding an excess of some of the salts of the heavy metals to precipitated proteins, the proteins may go into solution again. This is accounted for by the fact that the proteins undergo a certain degree of hydrolysis and break up into molecules which are smaller and soluble.

The "Salting Out" of Proteins by Means of Electrolytes.—It was already recognized by Denis (1856 (*a*)) and worked out in great detail by Kuhne (1886), Hofmeister (1887 (*b*)), and T. B. Osborne and his collaborators, that a great many salts have the power of throwing the proteins out of their solutions by precipitating them. These precipitated proteins, after removal of the salts, can be redissolved in distilled water, which makes the reaction a reversible one. It was further found that different proteins will be precipitated out by the different salts at definite points of salt concentration. This, therefore, enabled the above workers to fractionate the proteins and to obtain them in fairly pure state.

Kauder, working in Hofmeister's laboratory (1886), found that when small quantities of ammonium sulphate was added to blood serum, the precipitation of globulins commenced when the salt concentration reached 13 per cent of complete saturation, and ended when it reached 24.11 per cent. After the globulins were filtered off and fresh ammonium sulphate was added, no precipitation took place until the concentration of the ammonium sulphate reached 33.55 per cent, when the albumin fraction began to be precipitated. The latter precipitation was completed when the concentration reached 47.18 per cent.

Hofmeister further studied the relative influence of anions and the cations on the power of precipitating proteins. His results are summarized in the following table.

TABLE II

RELATIVE INFLUENCE OF ANIONS AND CATIONS ON THE PRECIPITATION OF PROTEINS

	Lithium	Sodium	Potassium	Ammonium	Magnesium
Sulphate	8.61	11.39	No pp.	13.39	15.93
Phosphate	Not investigated	11.69	13.99	16.57	Slightly soluble
Acetate	"	13.83	16.38	No pp.	No pp.
Citrate	"	14.42	17.07	21.99	Not investigated
Tartrate	"	15.11	17.08	25.05	"
Bicarbonate	"	No pp.	25.37	Not investigated	"
Chromate	"	21.22	25.59	No pp.	"
Chlorid	"	21.21	26.28	"	No pp.
Nitrate	Changes proteins	46.10	No pp.	"	"
Chlorate	Not investigated	58.82	"	Not investigated	Not investigated

From this it is evident that both the cation and the anions exert their influence on the precipitation of the proteins, and that the relative order of their efficiency is:

For Cations $Mg < NH_3 < K < NA < L$;
For Anions $CLO_3 < NO_3 <$ Bicarbonate $<$ Tartrate $<$ Citrate $<$ Acetate $< PO_4 < SO_4$

Coagulation and Denaturalization of Proteins

Because of the colloidal nature of the proteins, they are very susceptible to even slight changes. Solutions of albumin will fall out of solution merely on standing. A good many proteins will become coagulated even on small rise in temperature, while most proteins coagulate on boiling. This reaction in most instances is irreversible, i. e., the proteins become denaturalized and cannot be brought back into solution again.

Colloids that carry an opposite electrical charge may also coagulate the proteins.

The Salt Formation of Proteins

Until recently the question of salt formation of proteins was one of the most puzzling questions in biological chemistry. The proteins did

not seem to unite with the different ions in the same stoichiometrical ratios as they unite with crystalloids, and because of that, the proteins were credited with special "absorption" properties. These were attributed to all the colloids.

The recent researches of Jacques Loeb (1919-1921) seem to clarify the whole problem. He proved that the proteins, and perhaps all other amphoteric colloids, can exist in three states and that these states depend entirely upon the hydrogen ion concentration of the medium in which they are dissolved; that each protein has a critical point in the hydrogen ion concentration at which it does not dissociate and at which it is incapable of staying united with either anion or cation. At this point a protein like gelatin is almost completely insoluble, hence all the properties which are dependent upon the solubility of gelatin, like its osmotic pressure, viscosity, swelling and conductivity, are at a minimum. This point is known as the "isoelectric" point. For gelatin this isoelectric point lies at a hydrogen ion concentration of $C_H = 2.10^{-5}$ or $pH = 4.7$, for casein 4.7, for egg albumin 4.8, and for oxyhemoglobin at 6.8, and at these points we find the proteins to be almost inert bodies.

On either side of this isoelectric point the protein molecule dissociates in two different states. On the acid side, i. e., if the hydrogen ion concentration of a gelatin solution is raised and the pH falls below 4.7, the protein dissociates into a cationic state, carrying a positive electrical charge and capable of forming salts with anions forming protein chlorids, protein sulphates, etc. In this state the amino radical becomes chemically active, while the carboxyl, the other binding post of the protein molecule, is entirely inert.

On the other hand, if the hydrogen ion concentration of the solution is lowered and we have a rise in the pH above 4.7, the protein dissociates into an anionic state carrying a negative electrical charge and capable of forming salts with metals or cations, forming metal-proteinates, like sodium gelatinate, calcium albuminate, potassium caseinate, etc.

He further found that all proteins at their isoelectric points will abandon the chemical union they may have had with either anion or cation or other protein, and may be obtained in a state of high purity. He also found that for each given hydrogen ion concentration the proteins combine with the various anions or cations in definite stoichiometrical ratios similar to those of the crystalloids.

The Digestion of the Protein

During the process of mastication the proteins suffer only physical alteration by being broken up into smaller particles. The saliva contains

no enzyme which has any effect on the protein molecule by causing it to split into smaller compounds.

In the stomach we find an enzyme, pepsin, which is secreted in an inactive or zymogen state, and which is activated by the hydrochloric acid of the gastric juice. The activation of this enzyme can be accomplished also by organic acids, like oxalic, lactic and tartaric acids, or by inorganic acids like nitric, phosphoric and sulphuric.

The pepsin in acid solution has the power of splitting the protein molecule into simpler or "derived proteins." The longer digestion proceeds the smaller will be the size of the molecules of the "derived proteins" and the further these molecules will get away from the colloidal state and approach the crystalloidal. By means of fractional precipitation with ammonium sulphate or zinc sulphate; various fractions can be recognized, representing different stages in the digestion. These fractions are not definite chemical entities, but mixtures of what are known as meta-proteins, coagulated proteins, proteoses and peptones. Under no circumstances and no matter for how long pepsin is allowed to act on protein does its digestion lead to amino acid formation.

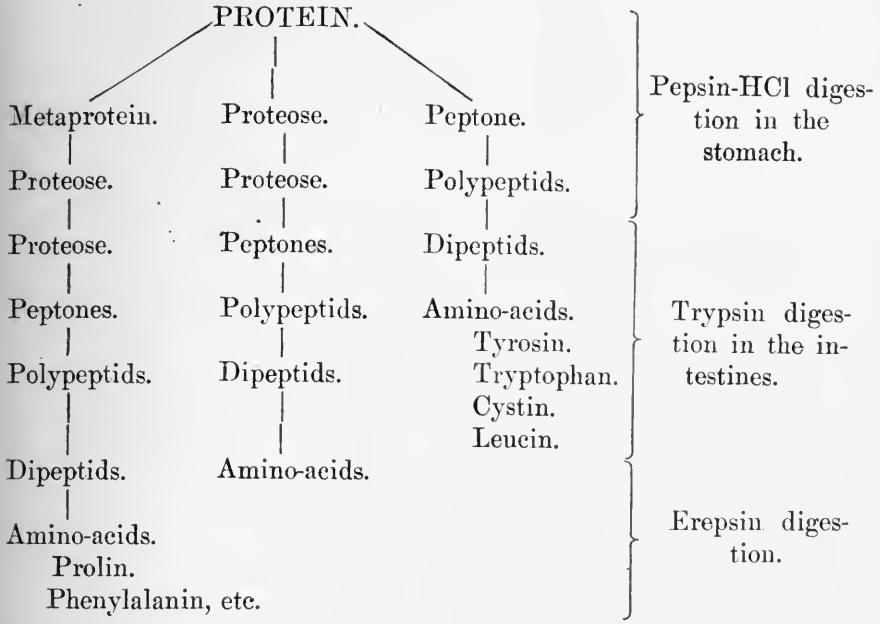
The hydrochloric acid plays an important part in the protein digestion. It causes a swelling of the protein, and a breaking up of the larger particles, converting it into a sort of gelatinous mass. The pepsin is thus enabled to make its way into the interior of the particles with much greater ease.

The products of protein digestion are passed on into the intestines, where they meet the secretions from the pancreas, liver and intestines. These render the mixture alkaline and thus prepare it for the action of trypsin, which acts only in alkaline mediums, and which is secreted by the pancreas in an inactive state, trypsinogen, and which is activated by the enterokinase of the succus entericus.

The trypsin acts on the peptic digestive products and also on the native proteins which have entered the intestines. The trypsin carries the digestion of the proteins mostly to the peptid stage, i. e., small chain compounds of amino acids, and to a considerable extent to the amino acid stage. Tyrosin, leucin, tryptophan and cystin are the amino acids that usually appear first in trypsin digestion.

When a protein is completely digested the products fail to give the biuret reaction, and when trypsin acts on protein long enough it carries the digestion to the stage where no biuret reaction is obtainable. E. Fischer and Abderhalden have shown that certain peptids exist which are composed of phenylalanin and prolin, which resist the action of trypsin and can only be broken up by another enzyme which is secreted by the intestinal glands and is known as erepsin. This enzyme has the power of breaking up all peptones into amino acids.

Schematic Illustration of the Digestion of Proteins in the Gastro-intestinal Canal



The above shows in a general way the scheme of protein digestion, and is reproduced to show that the protein molecule does not break up in an explosive manner, by which the whole molecule disintegrates, but that it takes place in stages, and that a large number of intermediary bodies are possible in the course of protein digestion.

The Absorption of Products of Protein Digestion from the Gastro-Intestinal Canal

From what was said above it is evident that digestion in the stomach does not proceed to the point where products are formed that are absorbable. Hence very little or no absorption of protein-digestion-products takes place normally (London-Abderhalden). If amino acids or peptones are introduced into the stomach they are absorbed with considerable rapidity (Folin and Lyman, 1912 (*a*)).

The greatest bulk of the absorption takes place from the intestines, from which the lower peptids and amino acids are absorbed with great rapidity, and carried by the blood stream to the various organs of the body.

Until about ten years ago it was believed that the amino acids were resynthesized into serum albumin and serum globulin while passing through the cells of the intestinal wall, and that these two products constituted the sole source from which all the body proteins were built up. The reason for that view was that while amino acids could be found in the intestines, none could be discovered in the blood stream. But since Van Slyke's introduction of his micro method for amino acid determination, this view had to be abandoned. Amino acids were then found to be present in the blood of fasting animals to the extent of 3 to 5 mg. per 100 c.c. of blood, and after a meal of meat the figures rose to 10 and 11 mgs. (calculated as amino acid nitrogen; Van Slyke, G. M. Meyer, 1913). Similar results were also obtained by Abderhalden and Lampe, 1912, and Folin and Denis, 1912 (*a*).

The Fate of Absorbed Amino Acids in the Blood

The amino acids, after they enter the blood stream, disappear from it fairly rapidly. This we know from various sources. First from the fact that there is but a very moderate rise in the amino acid nitrogen content of the blood during the height of digestion of a protein meal. Second from the results of the Van Slyke and Meyer's experiments (1913) which will be briefly summarized.

They found after injecting intravenously into a dog 1.90 grams of amino acid nitrogen obtained from digested casein, that the blood amino nitrogen rose from 4.05 mg. per 100 c.c. before the injection to 19.7 mg. one-half hour after the injection and came down to 7.85 mg. three and a half hours after the injection. At the same time they also found a rise in the urea nitrogen of the blood, and on examining the tissues of the body they found that their amino acid nitrogen content was increased considerably. Thus in one experiment, after injecting intravenously 4.06 grams of amino acid nitrogen they found that the blood amino nitrogen, thirty minutes after the injection, rose from 3.9 mg. per 100 c.c. to 45.2 mg. In the liver it rose from 31.5 to 93.5, in the muscles from 43 to 70 mg., while in the kidneys it rose from 45 to 106 mg.

From these experiments they concluded that there was a much larger amount of amino nitrogen retained in the tissues than in the blood, and that the tissues abstracted the amino nitrogen from the blood at a rapid rate so as to keep its concentration in the blood at a comparatively low and constant figure. They also concluded that the different tissues have different powers of absorbing amino nitrogen and that the amino acids are kept in the tissues, either by a process of mechanical absorption or in a loose chemical union with its proteins.

The Fate of Amino Acids in the Tissues

In the tissues the amino acids may undergo a number of changes, depending upon the requirements of the cells. They may undergo deamination by a process of hydrolysis in which the NH_2 is replaced by an hydroxyl radical, giving rise to the corresponding alcohol, forming hydroxylacids.

$$\begin{array}{lcl} CH_3 & & CH_3 \\ | & & | \\ CHNH_2 + HOH & \rightarrow & CHOH + NH_3 \\ | & & | \\ COOH & & COOH \end{array}$$

Alanin Water Lactic acid Ammonia

They may undergo deamination by a process of oxidation giving rise to the corresponding keto or oxy-acids.

$$\begin{array}{lcl} CH_3 & & CH_3 \\ | & & | \\ CHNH_2 + O & \rightarrow & CO + NH_3 \\ | & & | \\ COOH & & COOH \end{array}$$

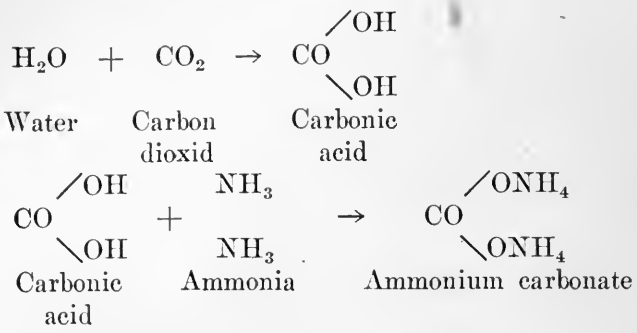
Alanin Oxygen Pyruvic acid Ammonia

They may be utilized by some cell in the synthesis of some organic body like a ferment, product of internal secretion, serum albumin, serum globulin, nucleoprotein, cell protein, etc.

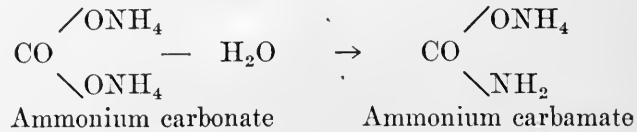
Urea Formation

During the process of deamination ammonia is set free. This ammonia is converted to its greatest extent into urea. We know that from the fact that if an ammonia salt is fed to an animal most of it is excreted in the form of urea (v. Schroeder, Salomon, Zaleski, Nencki and Pawlow), and also from the fact that if a single amino acid is fed to an animal, all of the nitrogen is excreted as urea (Levene and Kober, 1909). We also know that the liver is the organ which has the greatest power of converting ammonium salts into urea, and if amino acids are perfused through the surviving liver, urea is formed (Fiske and Karsner, 1913; Fiske and Sumner, 1914).

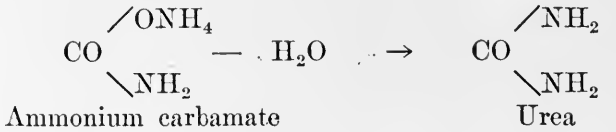
The reaction involved is no doubt the following: The ammonia as it is set free, combines with the carbon dioxid and water of the blood and tissue, forming ammonium carbonate.



The ammonium carbonate, on losing one molecule of water, is converted into ammonium carbamate.



which substance, on losing another molecule of water, is converted into urea.



In normal individuals, on normal diet, from 80 to 90 per cent of all the nitrogen is excreted in the form of urea, while about 3 to 5 per cent escapes in the form of ammonia.

Thus the nitrogenous element of the protein molecule plays a comparatively simple rôle in the physiological economy. As long as it is attached as an amino radical it forms one of the binding posts of the amino acid; it may enter into the formation of protoplasm, it may be built up into complex protein bodies, ferments, etc.; in other words it may play an important rôle in the life of cells. The moment it becomes dissociated it becomes dead matter, ready to be cast off and excreted in the urine.

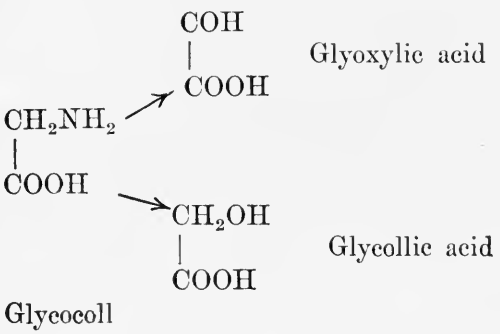
There is no heat liberated in the transformation of proteins to the amino acid stage, nor is there any heat liberated in the process of deamination or transformation of the ammonia into urea.

The Fate of the Non-Nitrogenous Fraction of the Amino Acids

The fate of the non-nitrogenous fraction of the amino acid in the animal body has been the subject of careful study during the past fifteen years, and the information obtained forms to-day one of the most interesting chapters in physiological chemistry.

Various methods have been employed in attacking this complex problem. The amino acids were fed to normal animals, phlorhizinized and depancreatized animals, and the results studied. They were perfused through surviving organs like liver, kidneys and muscles, and products of their metabolism sought for. They were incubated with different extracts of tissues, with ground up tissues, and their changes studied. Chemical substances that are related to the amino acids were fed to animals with the object of determining along which path the catabolism of the amino acid could possibly proceed.

In summing up all the work, the following conclusions may be drawn:[1] *Glycocoll* is completely converted into glucose (Ringer and Lusk, 1910). After deamination either glycollic acid or glyoxylic acid may be formed.

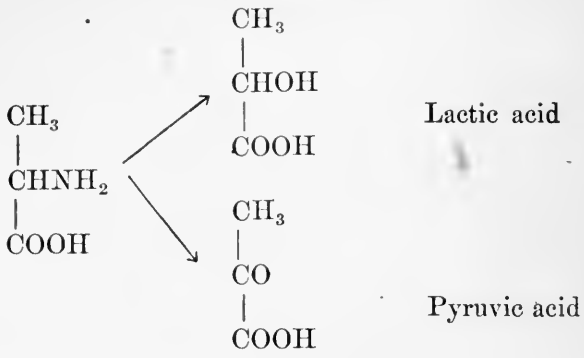


Neither one of these intermediary substances, however, has been found to give rise to sugar when administered to diabetic animals (Greenwald, 1918 (*g*); Ringer and Dubin, unpublished).

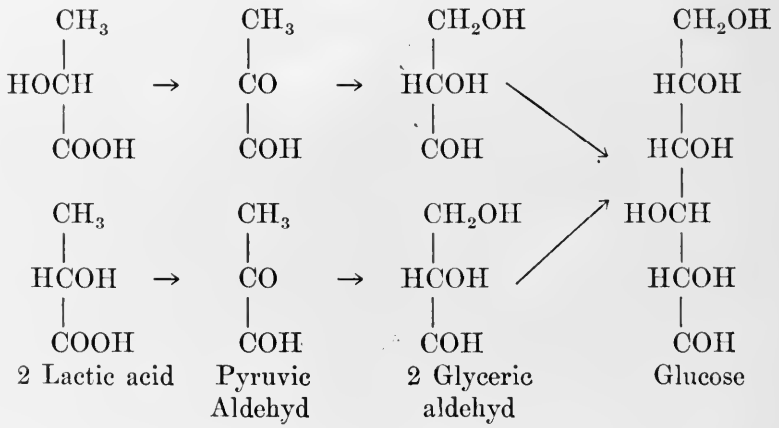
Glycocoll also plays a rôle in the formation of one of the bile salts, glycocholic acid, in which substance it exists combined with cholic acid. This is the first instance where a product of protein catabolism may be used by the cells in the synthesis of a definite compound that is essential for the welfare of the animal body.

Alanin is also completely converted into glucose. On deamination it may give rise to lactic or pyruvic acid.

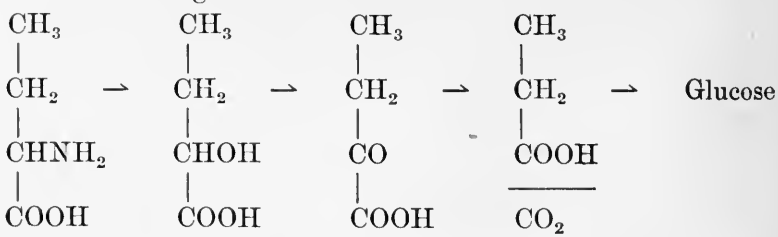
[1] This subject is thoroughly reviewed in the Third Edition of Lusk's "Science of Nutrition," pp. 184-207.



Of the two substances lactic acid is always and completely converted into glucose (Mandel and Lusk, 1906). Pyruvic acid, however, while it also goes over into glucose, does not do it in a quantitative way (Ringer, 1913 (*b*)). Dakin and Dudley assumed the transformation of lactic acid into glucose in the following way:

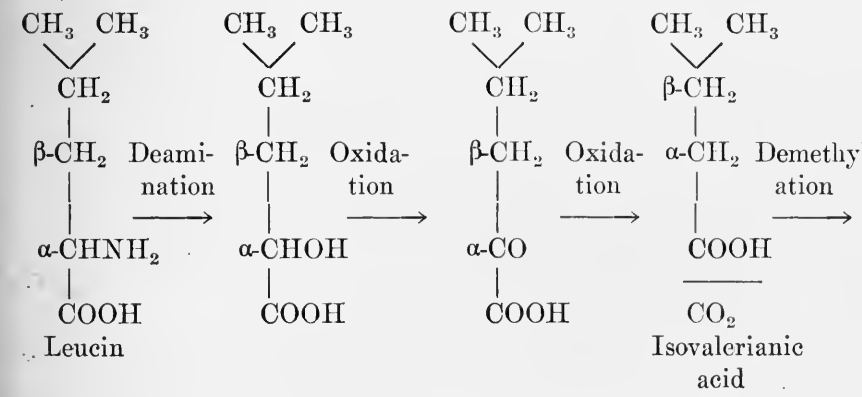


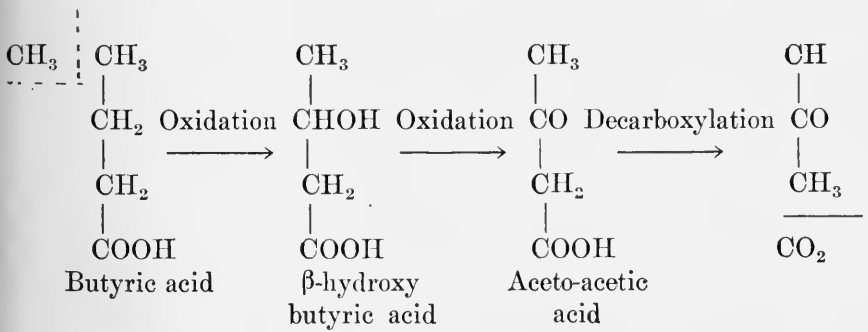
α-Amino butyric acid has not been investigated properly. In one single and uncorroborated experiment the giving of 10.3 grams of the substance to a phlorhizinized animal was followed by the excretion of 3.0 grams of extra glucose (Ringer, unpublished). On theoretical grounds this substance may be assumed to give rise to propionic acid, which was shown to be converted into glucose.



The fate of *valin* in the body is not definite. Dakin (1913) has found that it does not give rise to either glucose or acetone bodies. From *a priori* reasoning, and from experiences that were obtained with substances chemically related to it, one would have expected the transformation into glucose of three of its carbons.

The fate of *leucin* is definitely known. It does not give rise to any glucose, but gives rise to large amounts of β-hydroxybutyric acid and acetone. Baer and Blum, 1906 (*a*); Halsey, 1903; Dakin, 1913; Ringer, Frankel and Jonas, 1913 (*a*); Embden Salomon and Schmidt, 1906). The α-carbon is probably the first to suffer oxidation and the molecule becomes converted into iosovalerianic acid, which on demethylation is converted into butyric acid, and which on β-oxidation is converted into β-hydroxybutyric acid, aceto-acetic acid and acetone.

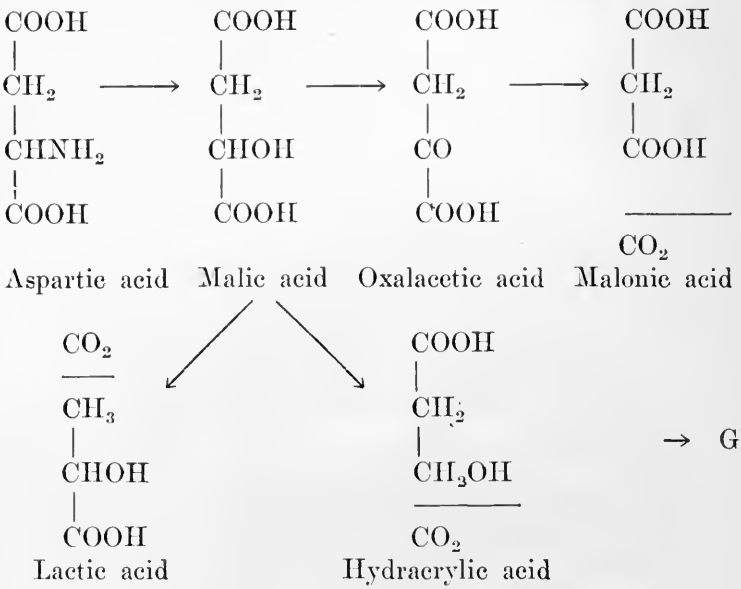




Isoleucin and *normal leucin.*—In Dakin's experiments (1913) we find an increase of 3.8 and 2.9 grams of glucose after administering 15 grams of isoleucin. Dakin is not inclined to consider that as conclusive proof that it is glucogenetic. From the structure of the normal leucin, however, one may assume the possibility of sugar formation. Normal valerianic acid may be formed after deamination and decarboxylation and this has been shown to be glucogenetic to the extent of three of its carbons.

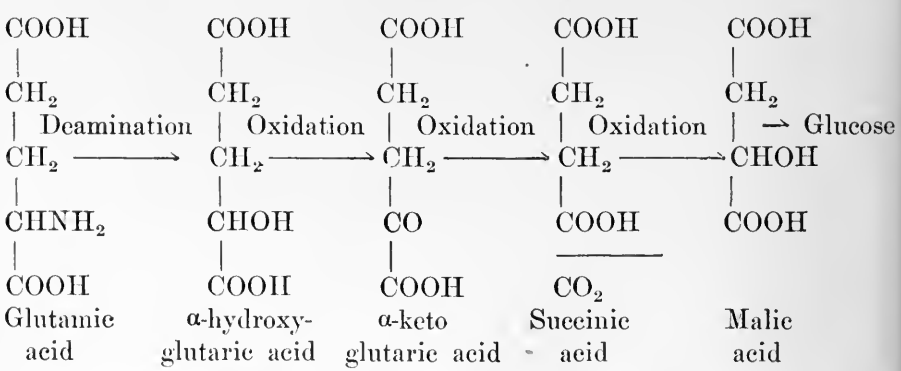
That normal leucin does give rise to glucose was demonstrated by Greenwald (1916 (*e*)).

Aspartic acid is definitely known to give rise to glucose to the extent of three of its carbons. (Ringer and Lusk, 1910; Ringer, Frankel and Jonas, 1913 (*b*)). It does not give rise to acetone bodies. In all probability the process of its conversion into glucose is the following:



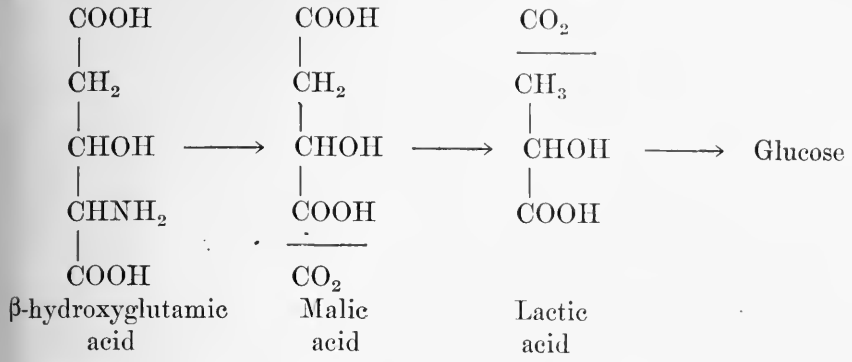
Glutamic acid is convertible into glucose to the extent of three of its carbons. It does not give rise to acetone bodies. (Lusk, 1908 (*a*); Ringer, Frankel and Jonas, 1913 (*b*)).

After deamination it probably passes through succinic and malic stages and then proceeds as indicated under aspartic acid.

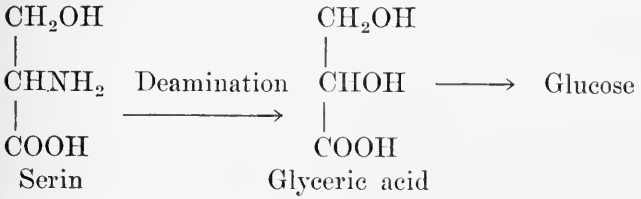


β-*hydroxyglutamic acid* is convertible into glucose to the extent as is glutamic acid. (Dakin, 1919).

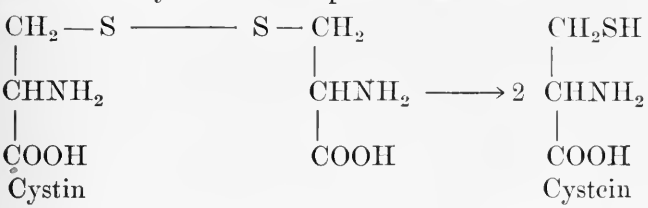
It does not give rise to acetone bodies. Its conversion into glucose in all probability is also through a malic acid stage.



Serin is converted into glucose, in all probability quantitatively. After deamination it may give rise to glyceric acid, which is convertible into glucose. (Dakin, Ringer and Lusk.)

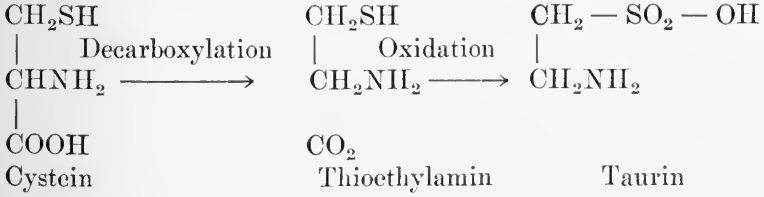


Cystin in the body is broken up into two molecules of cystein.



Cystein may undergo deamination and desulphurization yielding a three carbon compound which is completely converted into glucose (Dakin). The intermediary products are, in all probability, similar to those of serin.

Cystein to a small extent may also undergo decarboxylation, giving rise to thioethylamin, which on oxidation gives rise to taurin.

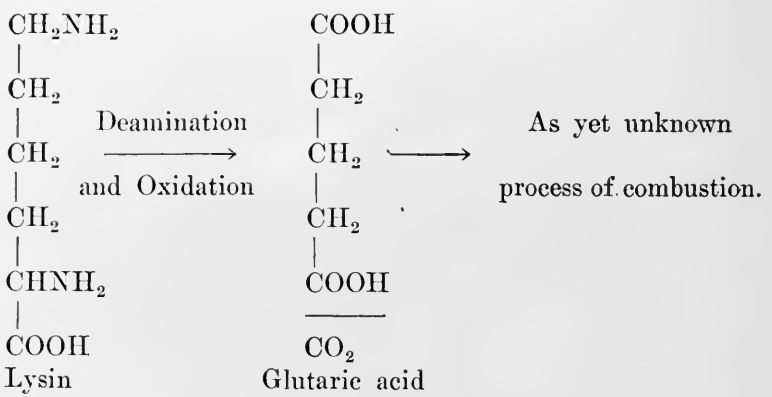


This taurin is used by the liver cells to combine it with cholic acid, forming taurocholic acid, which is one of the bile salts. This is therefore the

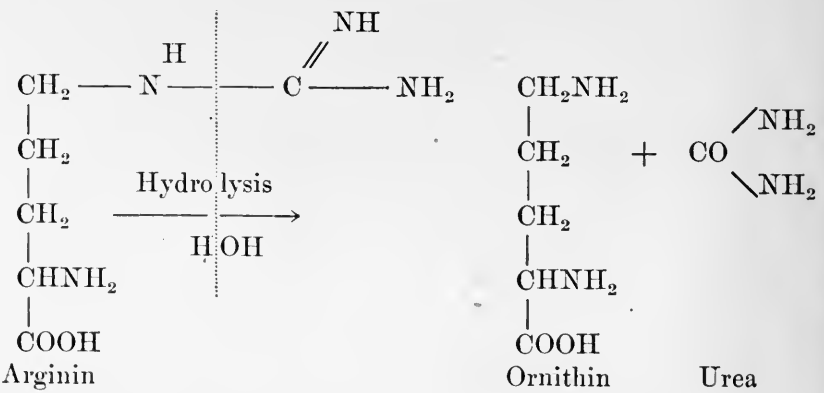
second illustration of the body's ability to utilize split products of protein for synthetic purposes. The hair and nails of animals are especially rich in cystin and no doubt a certain proportion of the cystein goes into the formation of these continually growing cells.

The greatest portion of the sulphur fraction of the cystein molecule is oxidized to a sulphate state and excreted in the urine in the form of inorganic salts. A small proportion of the oxidized sulphur combines with ethereal substances like cresol, phenol and indoxyl, probably for detoxicating purposes, and is excreted in the urine, while a third portion of the sulphur reaches the urine in an unoxidized form (neutral sulphur), probably in the form of taurin, small traces of cystein, sulphocyanid, etc.

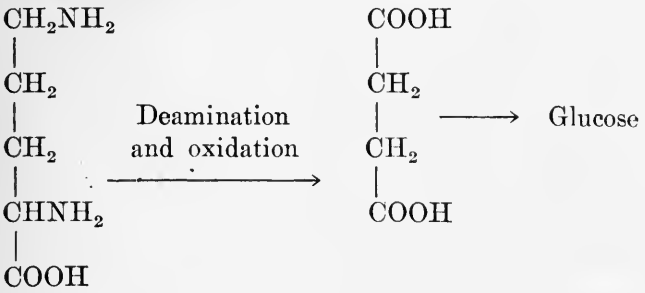
Lysin is completely burned in the body without leaving any clue as to the path of catabolism. It does not give rise to either glucose or acetone bodies in the intermediary stages. After deamination it may pass through a glutaric acid stage.



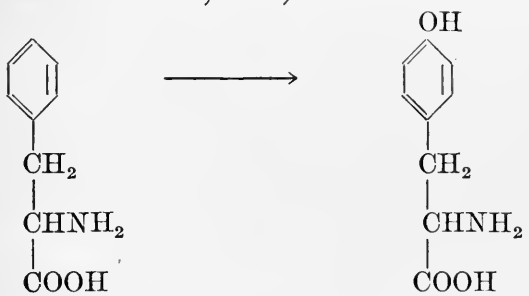
Arginin is first broken up into urea and ornithin. This is accomplished by a ferment arginase which is found in the liver, kidneys, intestinal mucous membranes, thymus and muscles. (Kossel and Dakin, 1904; and 1905; Otto Riesser, 1906 (*a*); Charles Richet, 1894 (*e*)).



Ornithin gives rise to glucose to the extent of three of its carbon atoms. (Dakin, Ringer, Frankel and Jonas, 1913 (*b*)). After deamination it probably passes through succinic acid stage.



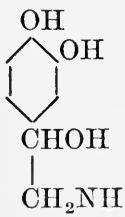
Phenylalanin and *tyrosin* have the same fate in the animal body. The former can be converted into the latter on perfusion through a surviving liver. (Embden and Balder, 1913).



Phenylalanin Tyrosin

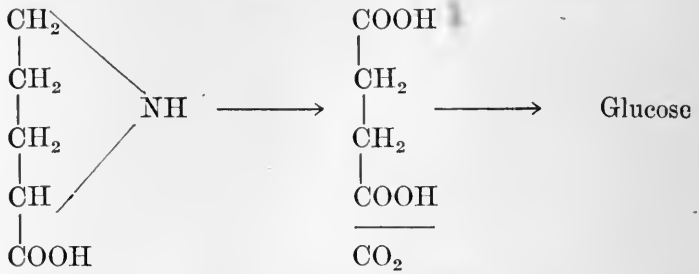
They are burned in the body, giving rise to acetone bodies in the intermediary metabolism (Ringer and Lusk; Dakin; O. Neubauer and Gross, 1910; E. Schmitz, 1910), but not to glucose.

Phenylalanin and tyrosin, as will be seen later, are indispensable amino acids (see page 000) i. e., an animal cannot maintain itself on proteins which do not contain these acids. When one views that fact in conjunction with the relationship that exists between the structure of the adrenalin molecule and tyrosin, one is justified in the conclusion that these two amino acids form the building material for adrenalin, even though we have no direct proof that such is the case. (Stolz, 1904; E. Friedman, 1905 (*a*); Abel and Crawford, 1897).



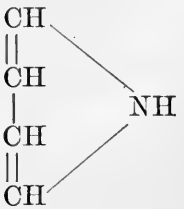
Adrenalin or Epinephrin

Prolin is burned in the body, passing through a glucose stage. Three of its carbons are convertible into glucose. (Dakin, 1913; Ringer, Frankel and Jonas.) In all probability, similar to glutaric acid, it passes through a succinic acid stage. It does not give rise to aceton bodies.



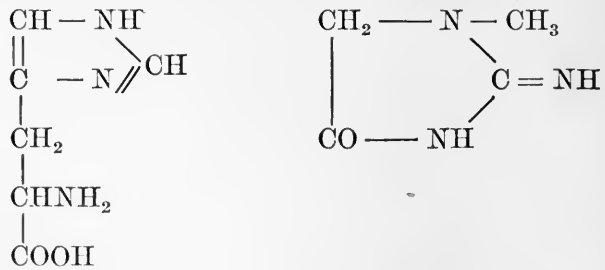
Prolin Succinic Acid

The fate of *oxyprolin* has not been worked out definitely. Both prolin and oxyprolin are intimately related to the pyrrol ring



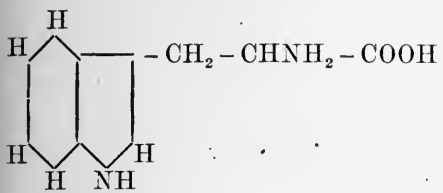
which forms the framework of hematin, one of the important derivatives of hemoglobin. Prolin is also found in a number of other coloring substances of the body, like in hair, the skin of dark races, melanins, etc. There can hardly be any question but that the body uses prolin and oxyprolin in the manufacture of the coloring materials.

The fate of *histidin* in the body is not clear. It does give rise to small amounts of glucose when fed to diabetic dogs and it also causes a slight rise in the acetone bodies formation when perfused through the surviving liver. Neither reaction, however, is definite nor conclusive. We must therefore wait for further research with this substance. Because of its structural relationship to creatinin, the possibility of its being the mother substance of creatinin has been suggested by Abderhalden.

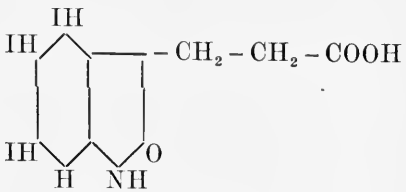


Histidin Creatinin

Tryptophan does not give rise to glucose nor to acetone bodies. It is one of the indispensable amino acids (see page 125). It may be considered the mother substance of thyroxin, the principal substance of the hormone of the thyroid gland (Kendal, 1919 (*c*)).



Tryptophan



Thyroxin

The fate of the amino acids in the body may be summarized in the following table:

TABLE III

FATE OF AMINO ACIDS IN THE ANIMAL BODY

Amino-acid	Gives Rise to Glucose	Gives Rise to Acetone Bodies
Glycocoll	+	—
Alanin	+	—
Valin	?	—
Leucin	—	+
Isoleucin	?	—
Normal Leucin	?	—
Aspartic Acid	+	—
Glutamic Acid	+	—
β-hydroxyglutamic Acid	+	—
Serin	+	—
Cystin	+	—
Lysin	—	—
Arginin (Ornithin)	+	—
Phenylalanin	—	+
Tyrosin	—	+
Prolin	+	—
Oxyprolin	?	—
Histidin	—	?
Tryptophan	—	—

Ten of the amino-acids are known definitely to give rise to glucose, and it is very possible that the four marked with the query may also give rise to glucose.

It was found by Lusk that dogs rendered diabetic by means of phlorhizin excrete 3.6 grams of glucose for every 6.25 grams of protein that they catabolize. Lusk and Mandel showed that severe human diabetics may excrete sugar in the same proportion, which means that from every one hundred grams of proteins catabolized, fifty-nine grams of sugar can be formed.

This does not yet complete the tale for three of the amino-acids give rise to not inconsiderable quantities of acetone bodies. Glucose and β-hydroxybutyric acid seem therefore to be the two important stations along

the highway of protein metabolism through which most of the amino acids have to travel while on their catabolic path.

Protein Metabolism

The studies of the metabolism of proteins date back to the days of Bischoff and Voit, in the middle of the last century, when it was recognized that the nitrogen excreted in the urine was derived from the catabolized proteins. Twenty-four hours are usually considered the unit of time for a protein metabolism experiment. Analysis is made of all the ingested food and of all the excreta. By determining the amount of nitrogen and multiplying that figure by 6.25, the protein factor is obtained. If the amount of nitrogen in the excreta, urine and feces, is equal to the amount of nitrogen in the food, we speak of the individual as being in a state of nitrogenous equilibrium. If there is less nitrogen excreted in the urine and feces than was ingested, the individual has stored some of the ingested nitrogen in the body. We therefore speak of his being in positive nitrogen balance. If, on the other hand, more nitrogen is excreted in the urine and feces than was ingested in the food, the individual must have lost nitrogen from his body, and we speak of that as his being in a negative nitrogen balance.

If an animal or human individual is allowed to fast for a long period of time, we find that nitrogen is excreted in the urine throughout the entire period of the fast up to the moment of death. This shows that protein destruction goes on in the body irrespective of any protein ingestion in the food. The amount of nitrogen excreted in the urine gradually diminishes in amount, in all probability due to the gradual depletion in the mass of the body proteins. Thus in the experiments by E. and O. Freund (1901) on Succi they obtained the following results:

TABLE IV

Day of Fast	Nitrogen in Urine	Day of Fast	Nitrogen in Urine
1	17.0	12	6.84
2	11.2	13	5.14
3	10.55	14	4.66
4	10.8	15	5.05
5	11.19	16	4.32
6	11.01	17	5.40
7	8.79	18	3.60
8	9.74	19	5.70
9	10.05	20	3.30
10	7.12	21	2.82
11	6.23		

Ringer and Dubin in experimenting on a dog weighing 17.0 kg. which fasted for forty-seven days, obtained the following results:

TABLE V

Day of Fast	Nitrogen in Urine	Day of Fast	Nitrogen in Urine	Day of Fast	Nitrogen in Urine
1	3.09	14	2.23	30	1.98
2	3.51	15	1.95	31	2.09
3	2.97	16	1.93	32	2.04
4	2.99	17	2.05	33	1.96
5	2.87	18	2.20	37	1.74
6	2.91	19	2.04	39	1.63
7	2.81	20	2.08	42	1.55
8	2.96	21	1.93	44	1.44
9	2.89	22	2.04	45	1.39
10	2.60	23	2.07	46	1.57
11	2.48	24	2.05	47	1.59
12	2.49	26	2.11		
13	2.27	28	2.04		

During starvation the various processes of life require a certain amount of fuel, which is derived from the body's own protein, carbohydrate (glycogen) and fat. If the necessary amount of carbohydrate and fat is supplied in the food, but no protein, the individual is kept in a state of "nitrogen hunger," and after five or six days the nitrogen excretion reaches the lowest level that is compatible with life. Landergren calls that the minimal nitrogen metabolism, whereas Rubner views that as representing the "wear and tear" quota.

Table VI gives the results of a number of experiments by different authors on the urinary nitrogen excretion in man when kept on carbohydrate and fat diet but free from protein.

From this table we see that 0.045 grams of nitrogen per kg. of body weight per twenty-four hours is the minimal amount on which the body can get along. It represents the "wear and tear" quota. This is an irreducible minimum. It corresponds to that part of the protein which cannot be replaced dynamically by any other foodstuff. It is that which is used for the formation of blood corpuscles, hormones, for the growth of hair, skin, nails, epithelial cells, etc.

If the carbohydrates are also removed from the diet and an isodynamic quantity of fat added, i. e., if an individual is given a diet free from both proteins and carbohydrates, with all the energy requirements supplied

TABLE VI

Day of Experiment	Nitrogen in Urine in Grams	Body Weight in Kg.	Nitrogen per Kg. of Body Weight	Author
10	3.8	64.0	0.0594	Folin
4	3.76	69.7	0.0539	Landergren
5	3.5	70.5	0.0497	Folin
4	3.04	62.4	0.0487	Landergren
5	2.7	55.7	0.0485	Folin
8	3.12	63.5	0.0480	Klemperer
7	3.34	71.3	0.0468	Landergren
7	2.42	57.5	0.0421	Roche
12	2.6	64.0	0.0406	Folin
8	2.51	65.0	0.0395	Klemperer
..	2.98	76.2	0.0391	Thomas
6	2.01	88.0	0.0319	Afklerker
7	1.84	58.0	0.0317	Siven
Average	2.897	66.6	0.0446	

in the form of fat, we also have a condition of nitrogen hunger and should expect the nitrogen excretion to be on as low a level as in the former case. But this is not so. With fat alone the protein metabolism rises to about double the "minimal" level. A typical experiment is that of Landergren's, which is tabulated here:

TABLE VII

Day	Diet	Nitrogen in Urine in Grams
1	Carbohydrate	8.91
2	Carbohydrate	5.15
3	Carbohydrate	4.30
4	Carbohydrate	3.76
5	Fat alone	4.28
6	Fat alone	8.86
7	Fat alone	9.64

On the fourth day the nitrogen reached the "minimal" level which would have continued thus had not the carbohydrates been replaced by fat in the diet. The carbohydrates have the power of sparing body protein to an extent which is not possessed by any other foodstuff. A diet made up so that half the calories are derived from carbohydrates and half from fat will give the same results as a diet consisting entirely of carbohydrates.

Landergren assumes that the reason why protein metabolism is higher when carbohydrate is absent from the diet is because a certain amount of protein is destroyed in order to maintain the sugar concentration of the blood, which is always kept at a definite level even during starvation. He designates that fraction of the protein metabolism as "glucose nitrogen." This fraction is equivalent approximately to 0.045 gram per kg. of body weight. Rubner and Cathcart have corroborated Landergren's findings, but do not agree with his interpretation.

The Question of Optimum Versus Minimum Protein Diet

When protein, in amounts corresponding to the "wear and tear" quota (0.045 grams per kg. of body weight), is added to a diet consisting of carbohydrates and fats sufficient to cover all the caloric requirements of an individual, he will not maintain nitrogenous equilibrium. For short periods of time, Siven (1900) was able to maintain himself in nitrogenous equilibrium on a level of 0.08 gram per kg. of body weight (almost double the "wear and tear" quota).

When Voit studied the nitrogen excretion of a number of individuals, who lived on general diets following the dictates of their appetites, he found the average excretion for a man of 70 kg. in body weight was 19 grams of nitrogen per twenty-four hours. He therefore came to the conclusion that for a normal man to keep himself in a good condition of nutrition a supply of 118 grams of protein per day was necessary. This corresponds to 0.271 gram per kg. of body weight or six times as much as the "wear and tear" quota.

These figures of Voit's were obtained after a statistical and not after a physiological study, and therefore caused considerable discussion and inquiry into their justification. The literature is filled with series of experiments, of shorter or longer duration, tending to prove that physical comfort and nitrogenous equilibrium can be maintained at much lower levels of protein metabolism than Voit's figures.[2] The most convincing of these are the ones reported by Chittenden and Hindhede. In a series of well-planned experiments on different individuals, representing different classes of workers, and carried on for a period of eight months, Chittenden (1904) obtained results which led him to the conclusion that normal adults can maintain themselves in nitrogenous equilibrium, and in good health, on levels from 0.093 to 0.171 gram of nitrogen per kg. of body weight,[3] with the greatest number maintaining equilibrium with 0.120 to 0.140 gram per kg., which is approximately three times the "wear and tear" quota. Taking the mean of the greatest number—0.130 grams per kg. of body weight—a man of 70 kg. would require 9.1 grams of nitrogen per day, which is equivalent to 57 grams of protein or one-half of Voit's figures.

Hindhede went a step further than Chittenden. His life for twenty-one years has been practically one continuous experiment. He and his family lived on an average of 50 grams of protein per person per day as the maximum. The nitrogen output in his urine kept close to 7.0 grams.

[2] For a complete review of the literature, see "Theorien des Eiweissstoffwechsels nebst einigen praktischen Konsequenzen derselben." L. B. Mendel. Ergebnisse der Physiologie, 1911, Vol. XI, pp. 418-525.

[3] Of the twenty-six men studied one maintained equilibrium on a level of 0.093, three between 0.100 and 0.109, three between 0.114 and 0.119, sixteen between 0.120 and 0.147, two at 0.150 and 0.151 and one at 0.171.

His children, who were brought up on this low protein diet, measured and weighed as much as others two years older, and possessed great endurance.

In another series of experiments his assistant lived for a period of 178 days on a diet consisting of 30.75 grams of protein (4.76 grams of nitrogen) with a total food supply of 3500 calories per day. Throughout the entire period he enjoyed excellent health and maintained his body weight.

During the period of the World War opportunity was afforded to study this problem on a large scale because of the forced reduction in protein ingestion by most of the people of the Central European empires.

Thus Lichtwitz (1917) reports the maintenance of nitrogenous equilibrium by citizens of Göttingen, living on 2400 calories and 64.9 grams of protein per day and weighing 70 kg.

Jansen (1917 (*a*)) carried on a series of experiments on thirteen individuals for periods of several months (March to May, 1917). They were engaged in light work and received 60.5 grams of protein, with carbohydrates and fats to make up a total energy supply of 1600 calories per day. On this diet they were unable to maintain either nitrogenous equilibrium or body weight.

The average loss per day was 0.28 kg. of body weight and 11.77 grams of protein (1.9 grams nitrogen). He then increased the carbohydrate and fat in the diet to the extent of 500 calories, i. e., they received the same amount of protein, but a total energy supply of 2100 calories. Doing the same amount of work, they were able to maintain nitrogenous equilibrium and body weight. The average weight of his subjects was 62.1 kg., the nitrogen ingested was 9.68 grams; hence the amount of nitrogen per kg. was 0.156 gram, or slightly above Chittenden's figures.

These experiments by Jansen prove definitely that it was not the low protein in the diet that was responsible for the loss in body weight and negative nitrogen balance, but the low caloric supply.

The question of optimum versus minimum protein supply in the diet of man cannot be answered on the basis of physiological experiments alone. In a great many instances, it is purely an economic question, and at the same time psychological factors and the influence of habit play a tremendous rôle.

Advocates of a low protein diet describe in glowing terms the psychic state of well-being when on a low protein diet, whereas the man accustomed to a full protein diet complains bitterly when forced to live on a restricted protein diet.

The consensus of opinion of most workers in this field seems to be that for a normal individual the ingestion of Voit's quota of 118 grams of protein per day (19 grams of nitrogen or 0.271 gram per kg. of body weight) is not objectionable, but offers no special advantage. Man can

get along perfectly well, grow to maturity, maintain his body weight and nitrogenous equilibrium on protein levels exactly one-half that of Voit's (that is, 0.130 gram per kg. of body weight) provided, of course, that he has a plentiful supply of dynamogenetic substances in the form of carbohydrates and fats to cover all of the body requirements.

From the mere fact that the hardest possible physical work is not associated with any increase in protein metabolism we may justly conclude that protein was not intended for dynamogenetic purposes. Its main function is to supply the "wear and tear" quota, "growth" quota with a reasonable surplus to allow for reserve and "factors of safety."

Sufficient data seem to have been gathered to date to show that 0.130 gram of nitrogen per kg. of body weight per twenty-four hours covers all of these requirements.

The Function of Protein in the Diet

Incomplete Proteins

The object of all food is to supply fuel, which, in the process of its catabolism, will yield energy to the cells. The use of protein serves a double function. While it may be used for dynamogenetic purposes, of far greater importance is its use in supplying the building stones of the protein to the body, i. e., the amino acids.

Originally it was believed that the peptones in the digested protein were the products that were resorbed and used for protein regeneration, and that the protein derived from the same species were utilized to greater advantage than proteins derived from foreign species (Michaud, 1909). It was further believed that in those peptones were nuclei of linked amino acids, which corresponded to those of the animals experimented upon, which made it possible for that animal to maintain equilibrium with a smaller amount of nitrogen derived from protein that was similar to its own protein. This conception, however, cannot stand, in view of the results obtained by Loewi (1902 (*a*)). He was the first to keep an animal on a diet consisting of carbohydrates and fats, with all the nitrogen that it required, supplied in the form of digested protein, that gave no biuret reaction, i. e., digested to the amino acid stage; proving that the animal body is capable of synthesizing its own protein from the elementary amino acids. These experiments have been repeated by Abderhalden and corroborated in a very convincing way. He not only cleared up the problem as to the possibility of synthesizing protein from the simple amino acids, but also introduced a new method for studying whether certain amino acids were dispensable or indispensable in the animal economy, and whether the body has the power of producing them

de novo or not. Abderhalden prepared a mixture of amino acids consisting of the following:

Amino Acid	Grams	Nitrogen Content in Grams
Glycocoll	5.0	0.9335
Alanin	10.0	1.5730
Serin	3.0	0.4002
Cystin	2.0	0.2330
Valin	5.0	0.5980
Leucin	10.0	1.0690
Isoleucin	5.0	0.5345
Aspartic Acid	5.0	0.5265
Glutamic Acid	15.0	1.4250
Phenylalanin	5.0	0.4245
Tyrosin	5.0	0.3370
Lysin (CO_3)	5.0	0.9585
Arginin (CO_3)	5.0	1.6090
Prolin	10.0	1.2170
Histidin	5.0	1.2980
Tryptophan	5.0	0.6860
	100.0 grams amino acids	= 13.87 grams nitrogen

Of this mixture he gave 25 grams per day to dogs whose nitrogen metabolism had been studied for periods of over seventy days. In addition to the amino acids, the dogs received daily 2.0 grams of predigested nucleic acids from thymus and yeast, 50.0 grams of a mixture of glycerin, oleic, stearic and palmitic acids, 20.0 grams of cholesterin, 50.0 grams of glucose, 5.0 grams of nitrogen-free bone ash and salts. This experiment lasted for eight days, and throughout the entire period the animal was able to maintain nitrogenous equilibrium and to retain its body weight.

The remarkable thing about this experiment is, that the animal received all of its food in its elementary form, and it had to synthesize not only its own protein, but also its fat.

This method of study is of great importance, because it enables us to make any kind of desirable mixture of amino acids, and also enables us to eliminate one or more amino acids and study their individual influences.

Thus he found that an amino acid mixture, containing no glycocoll or prolin, will enable an animal to maintain nitrogenous equilibrium. He also found that he can replace arginin by ornithin and obtain nitrogenous equilibrium. This proves that the body is capable of forming its own glycocoll and prolin and that the arginin union can be accomplished in the body.

He also proved that animals can utilize, with equal completeness, the amino acid mixtures obtained from the following digested proteins: casein, ox beef, milk powder, egg albumin, horse meat and dog meat.

Incomplete Proteins.—In the early studies of protein metabolism it was discovered that certain proteins could not maintain nitrogenous equilib-

rium. Gelatin was found to be one of these. No matter how much gelatin was administered to an animal, the animal would still continue to burn some of its own protein in addition. Krummacher (1896 *a*) went so far as to administer all of the animal's caloric requirements in the form of gelatin, but was not able to obtain nitrogenous equilibrium.[4] Various theories were advanced which were supposed to explain the reasons for this. Kauffman, in 1905, conceived the idea that the explanation may be found in the fact that gelatin lacks certain amino acids which may be indispensable to the animal organism. These are tryptophan, tyrosin and cystin. He therefore added small amounts of these to gelatin, carried out a series of experiments on man and dog, and found that nitrogenous equilibrium could be maintained under those circumstances. Abderhalden confirmed the experiments and went a step further. He took casein, digested it to the amino acid stage, and fed it to a dog for a period of seven days. During those seven days the dog gained 20.0 grams in weight and retained 0.12 gram of nitrogen per day. (See Table VIII, Section II.) During the succeeding six days the animal was given a corresponding amount of casein digest minus tryptophan. The animal lost 250.0 grams in body weight and lost nitrogen to the extent of 0.83 gram per day or 5.0 grams for the period of six days. (See Table VIII, Section III.) During the succeeding six days the animal was put back on its original diet. It regained 100.0 grams in weight and on the fourth day established nitrogenous equilibrium.

TABLE VIII

ABDERHALDEN'S EXPERIMENTS

I

DOG WAS FED 22 GRAMS OF PREDIGESTED DOG MEAT. EXPERIMENT SHOWS THAT NITROGENOUS EQUILIBRIUM AND BODY WEIGHT CAN BE MAINTAINED ON IT

Day	Diet	Body Weight in Grams	Nitrogen in Food	Total Nitrogen Excretion	Nitrogen Balance
1	22 grams of predigested dog meat	8250	2.50	2.27	+ 0.23
2	2 grams predigested nucleic acid	8245	2.50	2.32	+ 0.18
3	50 gr. glycerin-fat mixture	8240	2.50	2.32	+ 0.18
4	2 gr. cholesterin 50 gr. glucose	8245	2.50	2.32	+ 0.18
5	5 gr. bone ash salts	8240	2.50	2.32	+ 0.18
6		8250	2.50	2.35	+ 0.15
7		8250	2.50	2.26	+ 0.24
	Total	.....	17.50	16.16	+ 1.34
	Average	.....	.....	.....	+ 0.19

[4] For complete review of literature see Murlin, J. R., *American Journal of Physiology*, 1907, vol. 19, p. 285 and 1907, vol. 20, p. 234.

II

DOG WAS FED 18 GRAMS OF PREDIGESTED CASEIN. EXPERIMENT PROVES THAT NITROGENOUS EQUILIBRIUM AND BODY WEIGHT CAN BE MAINTAINED ON IT

Day	Diet	Body Weight in Grams	Nitrogen in Food	Total Nitrogen Excretion	Nitrogen Balance
41		8300	2.51	2.32	+ 0.19
42		8315	2.51	2.37	+ 0.14
43	18 grams of predigested casein	8320	2.51	2.42	+ 0.09
44		8310	2.51	2.20	+ 0.11
45	The rest as above	8320	2.51	2.40	+ 0.11
46		8320	2.51	2.41	+ 0.10
47		8320	2.51	2.42	+ 0.09
	Total		17.57	16.54	+ 0.83
	Average				+ 0.12

III

DOG WAS FED 22 GRAMS OF PREDIGESTED CASEIN, MINUS TRYTOPHAN. EXPERIMENT PROVES THAT ANIMAL LOSES ITS OWN NITROGEN BY BEING IN NEGATIVE NITROGEN BALANCE, AND ALSO LOSES IN BODY WEIGHT

Day	Diet	Body Weight in Grams	Nitrogen in Food	Total Nitrogen Excretion	Nitrogen Balance
48		8290	2.52	3.03	— 0.51
49		8300	2.52	3.07	— 0.55
50	22 grams of predigested casein minus tryptophan	8250	2.52	3.67	— 1.15
51		8210	2.52	3.65	— 1.13
52	The rest as above	8150	2.52	3.40	— 0.88
53		8070	2.52	3.30	— 0.78
	Total		15.12	20.12	— 5.00
	Average				— 0.83

IV

DOG WAS FED 20 GRAMS OF PREDIGESTED CASEIN PLUS TRYPTOPHAN. EXPERIMENT SHOWS THAT NITROGENOUS EQUILIBRIUM WAS REACHED ON THE FOURTH DAY. ANIMAL GAINED IN WEIGHT PROVING THAT TRYPTOPHAN IS AN ESSENTIAL AMINO ACID

Day	Diet	Body Weight in Grams	Nitrogen in Food	Total Nitrogen Excretion	Nitrogen Balance
54		8100	2.51	2.97	— 0.46
55		8125	2.51	2.87	— 0.36
56	22 grams of predigested casein plus tryptophan	8150	2.51	2.62	— 0.11
57		8150	2.51	2.48	+ 0.03
58	The rest as above	8150	2.51	2.46	+ 0.05
59		8170	2.51	2.51	0.00
	Total		15.06	15.91	— 0.85
	Average				— 0.14

V

DOG WAS FED 25 GRAMS OF THE AMINO ACID MIXTURES AS THE SOLE SOURCE OF NITROGEN SUPPLY. EXPERIMENT PROVES THAT NITROGENOUS EQUILIBRIUM AND BODY WEIGHT CAN BE MAINTAINED ON IT

Day	Diet	Body Weight in Grams	Nitrogen in Food	Total Nitrogen Excretion	Nitrogen Balance
60		8190	3.47	3.15	+ 0.32
61	25 grams of amino acids mixture	8200	3.47	3.27	+ 0.20
62		8200	3.47	3.40	+ 0.07
63		8200	3.47	3.58	— 0.11
64	The rest as above	8200	3.47	3.48	— 0.01
65		8200	3.47	3.49	— 0.02
66		8200	3.47	3.34	+ 0.13
67		8200	3.47	3.65	— 0.18
	Total	.....	27.76	27.36	+ 0.40
	Average	.....	.....	.....	+ 0.05

These experiments are of the utmost importance because they show the value of tryptophan in the physiological economy. They prove definitely that if an animal is kept on a diet free from tryptophan, the body has to burn its own protein to supply tryptophan to the cells that require it. (See the relationship between tryptophan and thyroxin, the active principle of the thyroid secretion, page 115.)

The proteins that do not contain all the indispensable amino acids are designated *incomplete proteins,* and the above experiment shows that a complete protein like casein can be made incomplete and cause it to be a non-sustainer of nitrogenous equilibrium by merely removing the tryptophan.

The study of the physiological values of the incomplete proteins and the influence of the individual amino acids have been carried on intensively for the past fifteen years.

In 1907 Hopkins and Willcock published a series of experiments on mice. They fed mice on a diet in which all the protein was supplied in the form of zein, a protein derived from maize, containing neither lysin nor tryptophan. The zein was mixed with carbohydrates, fats, lecithin and salts. In the first series of experiments five young mice were kept on this diet for seven days. On the seventh day they all showed the following losses in weight in per cent: 11.8, 17.6, 13.1, 23.2, 27.1.

As a control, four mice were kept on a similar diet, but the zein was replaced by a similar quantity of casein. On the seventh day the following increases in weight in per cent were recorded: 20.2, 21.8, 9.1, 21.0.

One of the mice of the first series was then given half of its protein in the form of zein and the other half in the form of casein, and it promptly began to gain in weight. After fifteen days it gained in weight to the extent of 46 per cent.

In another series of experiments, also on young mice, they studied the length of time the animals were able to survive the zein diet, and compared it with the controls that received two per cent of tryptophan in addition to zein. They found that of fifteen mice kept on the zein diet all died between the twelfth and twenty-second day, whereas of the fifteen on the zein plus tryptophan diet only three died before the twentieth day and all the others lived from twenty-four to forty-five days.

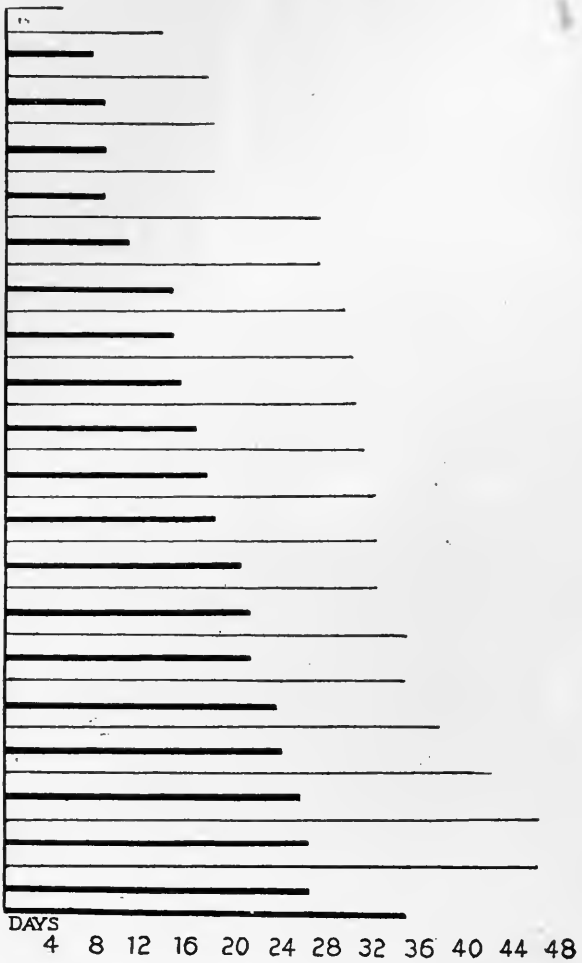


DIAGRAM I. Diagram constructed from the results of Hopkins' and Willcock's experiments 5, 6, 7. The heavy lines show the survival periods (in days) of twenty-one individual mice upon the zein diet with tyrosin. The light lines show the same for nineteen mice upon the zein diet with tryptophane.

There is therefore no question whatsoever but that the addition of tryptophan prolonged the time that the animals could live on zein. In studying the weights of the animals, however, they could not find any differences, i.e., the animals lost about as much in weight with the tryptophan as without it.

Osborne and Mendel took up the study of this subject on a very large scale (1911). They kept thousands of rats for periods of years, under absolutely controllable conditions of diet. They were thus able to study the influence of isolated food substances. They found the study of the changes in the body weight of the rat a most satisfactory index of the rate of growth. They selected the white rat because it is easily reared and cared for and because its food requirements are comparatively small. It also offers advantages because of the fact that it

thrives well on unvaried diets and maintains its health even though constantly confined to a cage. As the longevity of the white rat is about three years, they were able to study the influence of certain diets practically throughout the whole life time of the animal.

From hundreds of experiments published, four are selected here to illustrate the physiological value of some of the amino acids.

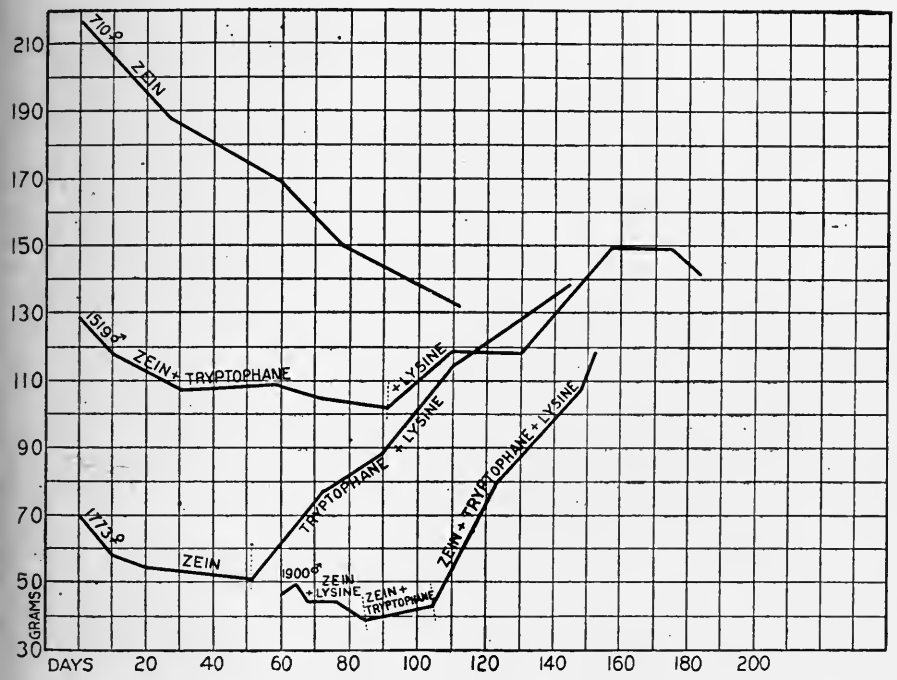


Diagram II illustrates graphically the result of Osborne and Mendel's experiments

Rat No. 710 was kept under observation from May 9, 1913, to September 5, 1913, a period of 120 days. During that period the animal lived on the following food mixtures: zein, 18.0 grams; protein-free-milk, 28.0 grams; starch, 27.0 grams; butter fat and lard, 27.0 grams; water, 15 c.c. The influence of this diet on the animal's body weight is presented in Table IX. Every one of the rats that was kept on this diet lost in weight. Rat 710 lost 39 per cent of its body weight in 120 days.

The experiment on Rat 1519 started on May 9, 1913, and ended Nov. 7, 1913. Between May 9 and August 8 it was kept on a mixture of zein, 16.92 grams, tryptophan, 0.54 gram, the rest as above. During this period the animal lost weight steadily, reaching the lowest level of 100.0 grams on August 8; 0.54 gram of lysin was then added to the diet. There followed an immediate gain in body weight, reaching the highest

TABLE IX

OSBORNE AND MENDEL'S EXPERIMENTS

RAT 710

Date	Diet	Body Weight in Grams	Date	Body Weight in Grams
1913			1913	
May 9	18 grams zein	218	July 11	168
13	28 grams protein-free milk	218	15	169
16	27 grams starch	212	18	170
20	27 grams butter fat and	205	22	159
23	lard	201	25	165
27	15 c.c. water	199	29	156
31		191	Aug. 1	157
June 3		194	5	147
6		186	8	154
10		184	12	148
13		187	15	150
17		183	19	143
20		180	22	144
24		175	26	137
27		180	29	138
July 1		177	Sept. 2	136
4		177	5	133
8		170		

RAT 1519

Date	Diet	Body Weight in Grams	Date	Body Weight in Grams
1913			1913	
May 9	16.92 grams zein	128	Aug. 15	109
13	0.54 gram tryptophan	122	19	109
16	The rest as above	123	22	113
20		117	26	118
23		115	29	118
27		114	Sept. 1	119
30		113	5	118
June 3		111	9	116
6		109	12	116
10		106	16	118
13		107	19	121
17		105	23	122
20		106	26	120
24		106	30	123
27		105	Oct. 3	129
July 1		106	7	135
4		107	10	142
8		102	14	150
11		102	17	150
15		104	21	148
18		103	24	147
22		103	28	148
25		102	31	149
29		102	Nov. 4	145
Aug. 1		100	7	141
5		100		
8		100		
12	0.54 gram lysin added	104		

TABLE X

RAT 1773

Date	Diet	Body Weight in Grams	Date	Body Weight in Grams
Sept. 23, 1913		70	Dec. 5, 1913	78
26.....		66	9.....	79
30.....	zein	61	12.....	81
Oct. 3.....		58	16.....	86
7.....		57	19.....	88
10.....		56	23.....	93
14.....		53	26.....	99
17.....		53	30.....	101
21.....		49	Jan. 2, 1914	105
24.....		49	6.....	112
28.....		48	9.....	113
31.....		46	13.....	115
Nov. 4.....		46	16.....	118
7.....		45	20.....	120
11.....		43	23.....	121
14.....		41	27.....	125
18.....	zein + tryptophan + lysin	47	30.....	130
21.....		57	Feb. 3.....	132
25.....		67	6.....	133
28.....		71	10.....	137
Dec. 2.....		76		

RAT 1900

Date	Diet	Body Weight in Grams	Date	Body Weight in Grams
Nov. 10, 1913		49	Jan. 1, 1914	55
13.....	zein + lysin	50	5.....	65
17.....		45	8.....	69
20.....		45	12.....	80
24.....		43	15.....	83
27.....		44	19.....	87
Dec. 1.....		40	22.....	90
4.....		39	26.....	91
8.....	zein + tryptophan	39	29.....	98
11.....		39	Feb. 2.....	99
15.....		41	5.....	103
18.....		42	9.....	113
22.....		43		
25.....		42		
29.....	zein + tryptophan + lysin	49		

point of 150 grams on October 14. It will be noticed in this experiment that on zein plus tryptophan the loss in weight was not as marked as on zein alone (rat 710). In many other experiments, Osborne and Mendel found that on zein and tryptophan the animals were able to maintain their body weight, but in no instance was an animal able to grow until after lysin was added. This led them to differentiate between maintenance and growth in nutrition. Without tryptophan, they showed, all animals

will lose in body weight quite sharply; after adding tryptophan, the curve of body weight becomes more horizontal. For an adult to just maintain his body weight is perfectly normal. But merely maintaining body weight for a child or growing animal is a decided abnormality. They have to grow, and growth does not occur until lysin is added to the diet.

The records of Rats 1773 and 1900 are corroborative of the first two.

From all the above data, the conclusion must be reached that the proteins in the dietary of all animals fulfill a series of functions which are not fulfilled by any of the other foodstuffs. They supply amino acids which the body itself cannot manufacture. Tyrosin, tryptophan and lysin are indispensable amino acids without which nutritional equilibrium cannot be established. Only plant cells have the power of synthesizing these.

For a protein, therefore, to be physiologically adequate, it must contain all of these amino acids and in sufficient quantities.

The study of the protein metabolism really resolves itself into a study of the metabolism of the amino acids. When we speak of a minimum protein requirement, we may in reality translate that into a minimum requirement of indispensable amino acids and the "wear and tear" quota may really represent that amount of protein which contains all the indispensable amino acids that are necessary for our maintenance.

The Influence of Protein on Metabolism

The Specific Dynamic Action of Protein

The final stage of all the metabolic processes in the animal body is one of oxidation, whereby energy is liberated in the form of heat. The amount of heat produced depends entirely upon the amount of material that is oxidized. When an animal is at rest and fasting, the oxidation processes are at a low ebb, the heat production is at a correspondingly low level. (We speak of its *basal metabolism.*) If the subject becomes more active, the oxidative processes and heat production increase in definite proportion, so that by doing fairly hard physical work the metabolism may reach a point double and triple the *basal* level.

A most remarkable phenomenon was observed by Voit in his early respiratory metabolism experiments. He found that even though at perfect physical rest, the heat metabolism of an individual increases after the ingestion of food; to a slight extent after carbohydrates, to a greater extent after fat, and to a most marked extent after protein. In other words, if we determine the starvation caloric requirements of an individual, and put him on a protein diet sufficient to cover those requirements, the individual's metabolism will increase as a result of ingesting the food and produce more heat than before.

In diagram III we have a graphic illustration of one of Lusk's experiments on a dog showing the influence of the ingestion of 1200 grams of lean meat on the metabolism of the dog. During the two hours before the meat ingestion, the heat production was 22 to 23 calories per hour. Within two hours after the meat ingestion the heat production went up to over 35 calories per hour, reached 44 during the third hour and remained

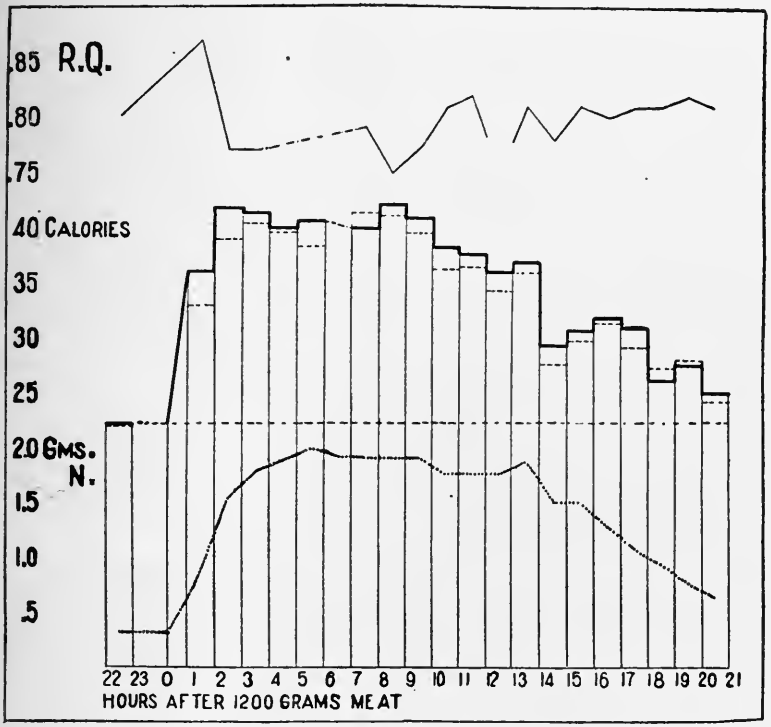


DIAGRAM III. Showing the respiratory quotient, the total metabolism determined by indirect (heavy black line) and direct (broken line) calorimetry as well as the nitrogen elimination (dotted line) during hourly periods after the ingestion of 1200 grams of meat.

at that high level for about eight hours, gradually coming down and reaching the basal level at the end of twenty-two hours. Ordinarily we notice increased heat production as a result of increased oxidation processes going on in the cells, as during periods of greater activity. The increase in Lusk's experiments corresponds to an increase in metabolism caused by violent exercise, and yet the animal was lying perfectly quietly and at rest.

Voit assumed that this marked increase in oxidation and heat formation was due to the cells being stimulated by the presence of food in the blood brought to them, and that the intensity of metabolism of a cell was a function of the quality and quantity of food material surrounding the

cell. The greater the amount of food brought to the cell, the more was it stimulated to catabolize it.

Rubner, Zuntz and Lusk have performed a great many experiments which may throw light on the cause of this increase in metabolism. Because of the specificity of each foodstuff to stimulate metabolism, Rubner called it the "specific dynamic action" of the foodstuffs. He believes that because the carbohydrates and fats are directly available to the cells for their nutrition there is therefore comparatively little increase in heat production after their ingestion. In the case of protein, however, it can contribute to the cell metabolism only in so far as it can give rise to glucose, and all the intermediary products which cannot go over into glucose are burnt, but their heat is given off as free heat and cannot be used by the cells.

Lusk proceeded to look for the cause of the specific dynamic action of the proteins along new lines. He realized that in order to analyze the action of protein on metabolism, one must take up the study of the influence of the individual amino acids, for it is they which come in intimate contact with the cells of the body. Then he reasoned thus: if Rubner's hypothesis be correct—that the fraction of the protein molecule which goes over into glucose is the one which contributes to the life of the cell, and that the fraction which does not go over is burned, giving rise to free heat—then amino acids like glycocoll and alanin, which are completely converted into glucose, should exert no specific dynamic influence at all, whereas glutamic and aspartic acids, which contribute only three of their carbons to glucose formation, should have a marked dynamic effect. Also, substances like leucin and tyrosin, which do not give rise to any sugar, should have a most pronounced dynamic effect.

Experiments not only failed to lend any support to Rubner's theory, but revealed just the contrary of what was expected. Glycocoll and alanin were found to possess a very pronounced power of stimulating metabolism and heat production. Leucin and tyrosin possess that power to a lesser extent, and aspartic and glutamic acids have none at all.

In another series of experiments Lusk found that the administration of 5.5 grams of glycocoll raised the heat production of a dog 7.3 per cent and 5.5 grams of alanin raised it 7 per cent. When he gave the two amino acids together there was a summation of influences and the heat production was raised 18 per cent. Ten grams of glycocoll caused a rise of 15.9 and 17.5 per cent in two successive experiments, and the giving of 20 grams of glycocoll caused a rise of 33.5 and 34.0 per cent in two experiments. Similar results were obtained after administering 20 and 30 grams of alanin.

These experiments prove beyond any question that the stimulus these amino acids exert is directly proportional to the amount of material administered.

Since glycocoll and alanin have been shown to be completely converted

into glucose in the diabetic animal, the question naturally presented itself, Will these amino acids exert a specific dynamic influence when given to a phlorhizinized diabetic animal?

In a series of experiments Lusk proved that in spite of the fact that all of glycocoll and alanin are converted into glucose and that none of it is oxidized, it still possesses the power of raising the heat production. The respiratory quotient in all cases remaining at the low diabetic level lends additional confirmation to the belief that none of these amino acids are oxidized in the diabetic animal.

From all this it becomes evident that the specific dynamic action of protein is a stimulus to metabolism which is given to the body by certain of the amino acids. It is not the result of these substances burning up as a sort of a bonfire, giving rise to free heat. They act as catalytic agents, spurring up the oxidative processes in the cells. The reaction is in reality much more "specific" than Voit and Rubner realized. It seems to reside in certain amino acids and not in others.

What the significance is of this spurring of metabolism by protein we do not know. All physiologists are agreed that the extra heat is wasteful and physiologically uneconomical. Advocates of the high protein diet seem to attach a great deal of importance to the sense of well-being a person experiences after a meal rich in protein, but whether a psychic state of well-being can be taken into consideration in determining physiological requirements and laws seems highly questionable. The drinking of wine and other alcoholic beverages certainly puts one in a psychic state of well-being, but no one will claim that this is sufficient evidence for its physiological requirement.

Nucleic Acids . . . . . *Walter Jones*

Chemical Part—Plant Nucleic Acid—The Fundamental Groups of Yeast Nucleic Acid—The Nucleotides of Yeast Nucleic Acid—The Nucleotide Linkages of Yeast Nucleic Acid—Inosinic Acid and Guanylic Acid—The Nucleosides of Yeast Nucleic Acid—Animal Nucleic Acid—The Partial Decomposition Products of Thymus Nucleic Acid—Physiological Part—The Physiological Decomposition of Nucleic Acid—The Formation of Uric Acid from Nucleic Acid—The Formation of Uric Acid from the Oxy-purines—The Formation of Oxy-purines from Amino-purines—The Physiological Destruction of Uric Acid—The Distribution of the Purine Ferments—The Enzymatic Decomposition of Combined Purines.

Nucleic Acids

WALTER JONES

BALTIMORE

Chemical Part

By a tedious manipulation it is possible to isolate from animal and plant tissues an organic acid, rich in both phosphorus and nitrogen, whose decomposition products are so far characteristic that not one of them is identical with any known decomposition product of a carbohydrate, a protein or a fat (Altman, 1889), (Osborne and Harris, 1902), (Kossel, (*a*), (*b*), 1879, 1880). The substance has been prepared from metamorphosed cell nuclei (Miescher, 1897), and as the amount of it that is obtainable from a tissue is proportional to the richness of the tissue in cell nuclei, it is properly regarded as a nuclear constituent and called nucleic acid.

Nucleic acid cannot be prepared sufficiently pure for chemical analysis, so that its chemical composition has not been directly found. This can be inferred, however, from a summation of its unique decomposition products. But chemical composition, physical properties and other considerations pertaining to nucleic acid as such, are matters about which, in the present state of our knowledge, physiology is little concerned. It is the decomposition products that are of importance, and these decomposition products are the same whether they are produced by chemical action outside of the body or by physiological agents present in the tissues; so that a discussion of the chemical decomposition of nucleic acid will disclose its metabolic possibilities.

Plant Nucleic Acid

It was formerly supposed that a multiplicity of nucleic acids exist, and that each tissue contains its individual substance of this class. But without entering into the obscure and contradictory older contributions, it is safe to state that everything known is in accordance with the assumption that there are two, and only two, nucleic acids in nature: one is obtainable from plant tissues (yeast nucleic acid) (Kossel, 1893), and the other is obtainable from animal tissues (thymus nucleic

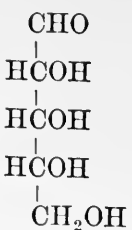
acid). (Kossel and Neuman (*a*)(*b*)(*c*), 1893, 1894.) It will, therefore, be necessary and sufficient to examine two nucleic acids in order to get a knowledge of them all.

The Fundamental Groups of Yeast Nucleic Acid.—When yeast nucleic acid is heated for a short time with very dilute sulphuric acid, part of its molecule easily undergoes hydrolysis with the formation of pentose, phosphoric acid and two purine derivatives (guanine and adenine). But when the nucleic acid is submitted to severe hydrolysis by heating with stronger sulphuric acid in an autoclave at 160°, a second part of its molecule is decomposed with the formation of pentose and phosphoric acid as before, but in addition, two pyrimidine derivatives (cytosine and uracil). So that by hydrolysis with mineral acid in one way or another, yeast nucleic acid produces six substances.

1. *Phosphoric Acid*
2. *Pentose*
3. *Adenine*
4. *Guanine*
5. *Cytosine*
6. *Uracil*

These six substances constitute the fundamental groups of which yeast nucleic acid is composed, and as will be seen later, the same six substances are formed when yeast nucleic acid is decomposed by physiological agents. One of them is so simple as to require no treatment; the other five should be discussed.

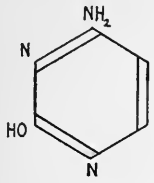
Pentose.—There are theoretically possible, eight aldo-pentoses of the formula $C_5H_{10}O_5$. The substance which is obtained from yeast nucleic acid is that one of the eight possibilities that has the geometrical configuration called *dextro-ribose.* (Levene and Jacobs (*c*)(*g*)(*h*), 1909, 1909, 1910.)



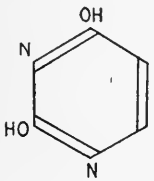
This configuration is unique, being found very rarely in nature, and it probably has great physiological significance, but at present we can only refer d-ribose to the general metabolism of the carbohydrates; in which case it does not properly fall into a discussion of nucleic acids.

The Pyrimidine Derivatives.—Both cytosine (Kossel and Neuman (*a*)(*b*), 1893, 1894), (Kossel and Stendel (*a*)(*b*), 1902, 1903) and uracil

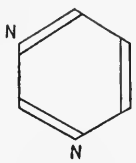
(Ascoli, 1900) are chemically referable to hypothetical pyrimidine. Cytosine is 6-amino-2-oxypyrimidine and uracil is 2-6-dioxy-pyrimidine.



Cytosine
$C_4H_5N_3O$



Uracil
$C_4H_4N_2O_2$



Pyrimidine

The two substances are corresponding oxy- and amino-compounds, so that one may pass into the other by deaminization

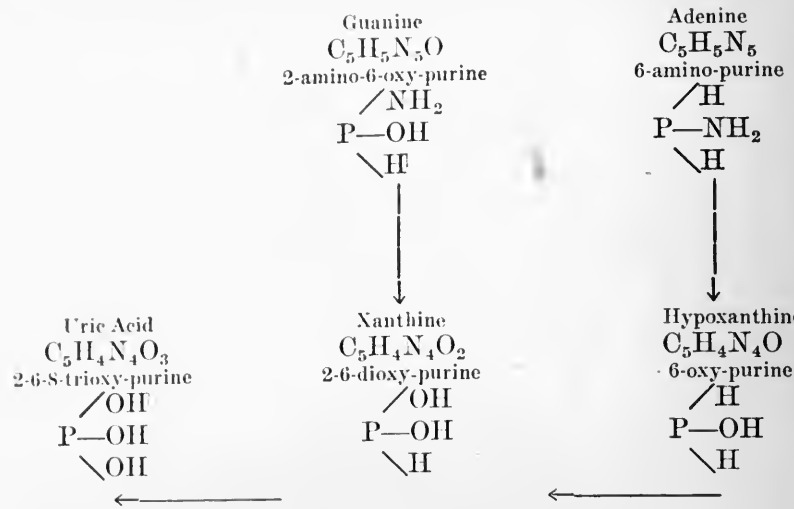
$$C_4H_2N_2{<}^{OH}_{NH_2} + H_2O = C_4H_2N_2{<}^{OH}_{OH} + NH_3$$

Cytosine Uracil

In fact, cytosine can easily be converted into uracil, and will be so converted in a laboratory manipulation of the material unless precautions are taken against it. The relation of the two substances to each other suggests the possible metabolic conversion of one of the compounds into the other by the deaminizing ferments of the tissues. This is, of course, possible, but the transformation has not been shown either by an organism or by a tissue extract. In fact, very little is known about the metabolism of the pyrimidine derivatives, so that of the six fundamental decomposition products of yeast nucleic acid, physiological interest is directed almost exclusively to the purine derivatives.

The Purine Derivatives.—By hydrolysis of yeast nucleic acid with dilute mineral acid, it is possible to obtain only the two amino-purines, guanine and adenine; but in studying the metabolism of these two, it is necessary to consider three other purine derivatives, viz., hypoxanthine, xanthine and uric acid. The chemical relation of these five substances to one another is shown in the following arrangement, in which the purine ring is represented by the letter P.

[In this article, purine formulas are used to which the physician may not be accustomed and a word of explanation may not be superfluous. There are two tautomeric formulas for purine derivatives (enol formulas and ketol formulas) which are not chemically distinguishable from each other. One of these formulas is almost universally (but arbitrarily) used by chemists and physiologists. The other formula has been adopted in the following pages for its exceeding convenience in dealing with the problems under consideration.]

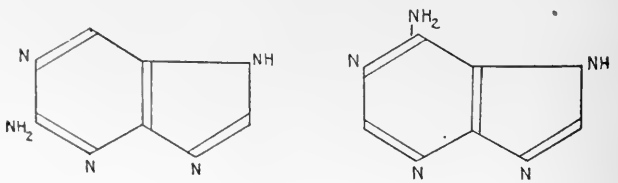


Guanine and adenine are referred to collectively as the amino-purines; xanthine and hypoxanthine as their corresponding oxy-purines. The amino-purines may easily be converted into the oxy-purines by a deaminizing agent (nitrous acid).

$$\underset{\text{adenine}}{C_5H_3N_4(NH_2)} + H_2O = \underset{\text{hypoxanthine}}{C_5H_3N_4(OH)} + NH_3 \quad \text{(Kossel (}c\text{), 1886)}$$

$$\underset{\text{guanine}}{C_5H_3N_4O(NH_2)} + H_2O = \underset{\text{xanthine}}{C_5H_3N_4O(OH)} + NH_3 \quad \text{(Strecker, 1858)}$$

and it will be seen that these transformations are actually brought about by deaminizing ferments present in the tissues. But guanine and adenine cannot be directly converted into one another. The one has its amino-group in position two; the other, in position six.



By oxidation, hypoxanthine could conceivably be changed into xanthine

$$C_5H_4N_4O + O = C_5H_4N_4O_2$$

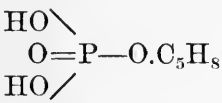
and this in turn could be oxidized to uric acid

$$C_5H_4N_4O_2 + O = C_5H_4N_4O_3$$

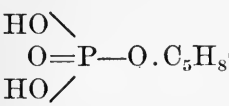
but it would be necessary to introduce the first oxygen atom into position two, and the second, into position eight. While no chemical oxidizing agent has been found that can effect this selective oxidation, oxidizing ferments are present in the tissues that can direct the oxygen atoms into their proper positions, and bring about the conversion of hypoxanthine successively into xanthine and uric acid.

The converse reactions which involve the withdrawal of oxygen can be effected in the laboratory. Uric acid has been successively reduced to xanthine and hypoxanthine. (Sundwick, 1911.)

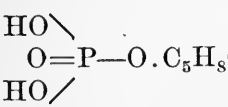
The Nucleotides of Yeast Nucleic Acid.—The older investigators knew that by mild acid hydrolysis. nucleic acid is partly split up, setting free part of its phosphoric acid, part of its carbohydrate and all of its purine bases; but that the remainder of its phosphoric acid and carbohydrate, together with its pyrimidine compounds, are set free only after most violent methods of hydrolysis. It was therefore natural to assume that nucleic acid is composed of four "complexes," all of which produce both phosphoric acid and carbohydrate, but each "complex" produces a different one of the four nitrogenous compounds. The two purine "complexes" evidently undergo hydrolysis with ease, while the two pyrimidine "complexes" are very stable. If the term "nucleotide" be substituted for the term "complex," this becomes essentially the modern nucleotide theory of the constitution of nucleic acid. This theory was originally proposed on the speculative grounds as outlined above, before any nucleoside or nucleotide had been prepared from nucleic acid; but it has recently received firm experimental support by the preparation from yeast nucleic acid of the four assumed nucleotides



Guanine Nucleotide (Jones and Richards, 1914) (Read, 1917)



Cytosine Nucleotide (Thannhauser and Dorfmüller (*a*) (*b*), 1918, 1919)



Adenine Nucleotide (Jones and Kennedy, 1918)

Uracil Nucleotide (Levene (*d*), 1919)

They are crystalline dibasic acids which closely resemble phosphoric acid in their acidic conduct. They form crystalline dibrucine salts which differ from one another in their solubilities, thus making possible the purification of the nucleotides and their separation from one another.

The two purine nucleotides easily undergo acid hydrolysis, giving rise to phosphoric acid pentose and purine base: but the pyrimidine nucleotides are very stable, and must be treated severely before hydrolysis is effected. This explains the conduct of nucleic acid toward hydrolytic agents.

It will be seen that a thermostable physiological agent (a ferment?) is present in the pancreas, which at the body temperature causes a decomposition of yeast nucleic acid into its four component nucleotides.

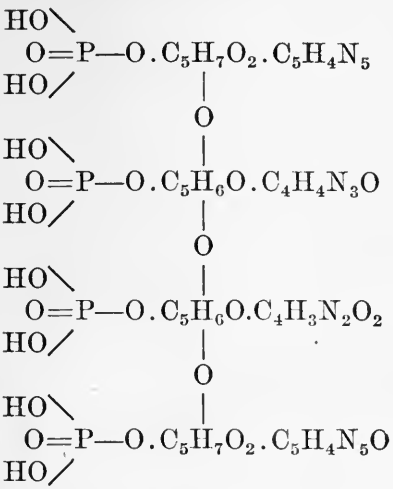
The Nucleotide Linkages of Yeast Nucleic Acid.—It has been pointed out that the work of the earliest investigators indicated the nucleotide structure of yeast nucleic acid. But this work gave no suggestion of the points where the four nucleotides are united to one another in yeast nucleic acid, or in other words, the location of the nucleotide linkages. The location was later assumed, without any evidence, to be through the phosphoric acid groups, but this assumption is not correct. The nucleotide linkages involve neither the phosphoric acid groups, nor purine groups, and probably not the pyrimidine groups. This conclusion is based principally upon the following.

I. The conversion of yeast nucleic acid into simpler nucleotides is not attended by an increase in acidity. (Jones (*e*), 1920.) There would be a marked increase in acidity if the nucleotide linkages involved the phosphoric acid groups.

II. The laws governing the liberation of phosphoric acid from the nucleotides are the same, whether the nucleotides are free or combined in nucleic acid. The same is true for the purines, and also for the pyrimidines, so far as experiments with the latter are possible. (Jones (*d*) 1920.)

If the nucleotide linkages involve neither the phosphoric acid groups, the purine groups nor the pyrimidine groups, they can only involve the carbohydrate groups. Nucleic acid should therefore probably have the following formula which represents the substances as a polysaccharide.

[It should be noted that this formula is arrived at by exclusion and is intended primarily to indicate the points at which the nucleotide linkages *do not exist.*]



Inosinic Acid and Guanylic Acid.—These two substances were known to be constituents of animal tissues before the constitution of yeast nucleic acid had been proposed, and one of them was the subject of considerable discussion because it was looked upon as a peculiar nucleic acid; but both are purine nucleotides of the class that has been discussed.

Inosinic Acid.—This substance was discovered by Liebig (*a*) (1847) in meat extract, and is now known to be a constant and characteristic constituent of muscle tissue. By mild hydrolysis with mineral acid, it easily decomposes into phosphoric acid pentose and hypoxanthine (Bauer, 1907) (Newberg and Brahn (*a*)(*b*) 1907, 1908).

$$C_{10}H_{13}N_4PO_8 + 2H_2O = H_3PO_4 + C_5H_{10}O_5 + C_5H_4N_4O$$

The substance is marked by the pentose, which is identical with the pentose of yeast nucleic acid. The muscles of *animals* contain a nucleotide that is unmistakably related to *plant* nucleic acid. (Levene and Jacobs (*b*) 1909.) The relation is not one of identity, for inosinic acid produces hypoxanthine, where the nearest nucleotide of yeast nucleic acid produces adenine. If the one nucleotide originates from the other (the plant food of the animal), deaminization of the adenine group must occur somewhere.

Inosinic acid occupies a unique place in a discussion of yeast nucleic acid, for, though it is not a nucleotide of yeast nucleic acid, it is the first nucleotide whose constitution was solved, and the method of solution was afterward applied to the purine nucleotides of yeast nucleic acid. Inosinic acid is composed of three groups, and gives rise to three, and only three substances by acid hydrolysis, viz., phosphoric acid, pentose and hypoxanthine. Theoretically, any one of the three groups may be the central group connecting the other two.

$$\begin{array}{l} HO\diagdown \\ \quad O{=}P{-}O.C_5H_8O_3.C_5H_3N_4O \\ HO\diagup \end{array} \qquad (1)$$

$$C_5H_9O_4.O{-}\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}{-}O.C_5H_3N_4 \qquad (2)$$

$$C_5H_9O_4.C_5H_2N_4.O{-}\overset{\overset{OH}{\diagup}}{P{=}O}{\underset{OH}{\diagdown}} \qquad (3)$$

Inosinic acid is a dibasic acid, so that formula (2) is excluded. It sets free its hypoxanthine much more easily than its phosphoric acid. This would not be possible if the hypoxanthine group were internal to the phosphoric acid group; so that formula (3) is excluded. The correct formula (1) remains. The order of the groups in adenine nucleotide and guanine nucleotide has been proven in a similar way. (Jones (*d*) 1920) (Jones and Read, 1917.)

Of the greatest interest is the hydrolytic action of ammonia on inosinic acid under pressure. When so treated, the substance loses its phosphoric acid completely, while the linkage between the pentose and hypoxanthine groups is not disturbed, so that a phosphorus-free compound is produced called inosine. (Levene and Jacobs (*a*) 1909.)

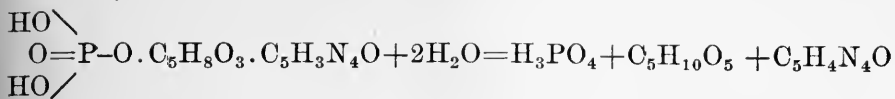
$$\begin{array}{l} HO\diagdown \\ \quad O{=}P{-}O.C_5H_8O_3.C_5H_3N_4O \\ HO\diagup \end{array} + H_2O = \begin{array}{l} HO\diagdown \\ \quad O{=}P{-}OH \\ HO\diagup \end{array} + C_5H_9O_4.C_5H_3N_4$$

Inosine is typical of a class of compounds called nucleosides. As from inosinic acid, so also from any nucleotide a nucleoside may be prepared by hydrolysis with ammonia.

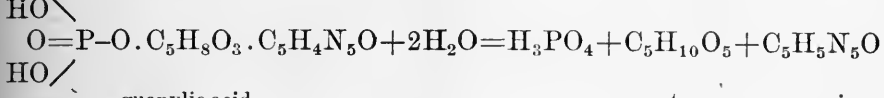
Guanylic Acid.—This substance is a strict analogue of inosinic acid. It is found in animal tissues (principally the pancreas) and doubtless originates from the plant food, for it is identical with guanine nucleotide prepared from yeast nucleic acid. By mild acid hydrolysis, it splits easily into phosphoric acid pentose and guanine, setting free the guanine much more rapidly than the phosphoric acid. As with inosinic acid, guanylic acid loses its phosphoric acid and forms its nucleoside by hydrolysis with ammonia.

The chemical analogy between the two nucleotides is shown in the following equations:

I. By acid hydrolysis

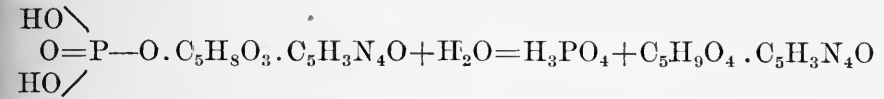


inosinic acid — pentose — hypoxanthine

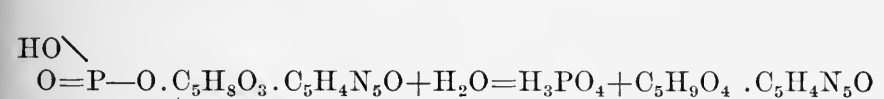


guanylic acid — pentose — guanine

II. By hydrolysis with ammonia



inosinic acid — inosine



guanylic acid — guanosine

Thus, inosinic acid (from muscle) is hypoxanthine nucleotide, or deaminized adenine nucleotide, one of the purine nucleotides of plant nucleic acid.

Guanylic acid (from pancreas) is guanine nucleotide, one of the nucleotides of plant nucleic acid.

The plant origin of both nucleotides is shown by the identity of their characteristic pentose (d-ribose).

The Nucleosides of Yeast Nucleic Acid.—When yeast nucleic acid is submitted to mild alkaline hydrolysis (as with ammonia at 110°), it easily decomposes into its four component nucleotides. But when alkaline hydrolysis of the nucleic acid is effected at higher temperatures (as with ammonia at 150°), the four nucleotides first formed lose their phosphoric acid, and are converted into the corresponding four nucleosides. (Levene and Jacobs (*e*) (*f*) (*h*), 1909, 1910.)

[The logical order of treatment is from nucleotides to nucleosides but this is not the order of discovery as the nucleosides were discovered first. A long period of time elapsed between Kossel's discovery of the fundamental decomposition products of nucleic acid and Levene's discovery of the first partial decomposition products (the nucleosides). The isolation of the nucleotides by Jones and by Thannhauser came afterwards.]

The four nucleotides	The four nucleosides
$HO\diagdown$ $O{=}P{-}O\,.\,C_5H_8O_3\,.\,C_5H_4N_5$ $HO\diagup$ adenine nucleotide	$C_5H_9O_4\,.\,C_5H_4N_5$ adenine nucleoside
$HO\diagdown$ $O{=}P{-}O\,.\,C_5H_8O_3\,.\,C_4H_3N_2O_2$ $HO\diagup$ uracil nucleotide	$C_5H_9O_4\,.\,C_4H_3N_2O_2$ uracil nucleoside
$HO\diagdown$ $O{=}P{-}O\,.\,C_5H_8O_3\,.\,C_4H_4N_3O$ $HO\diagup$ cytosine nucleotide	$C_5H_9O_4\,.\,C_4H_4N_3O$ cytosine nucleoside
$HO\diagdown$ $O{=}P{-}O\,.\,C_5H_8O_3\,.\,C_5H_4N_5O$ $HO\diagup$ guanine nucleotide	$C_5H_9O_4\,.\,C_5H_4N_5O$ guanine nucleoside
H OH H OH H OH	

The four nucleosides were prepared from yeast nucleic acid before the nucleotides were known, and thus gave experimental probability to the tetra-nucleotide structure of yeast nucleic acid, which up to that time had been simply speculative.

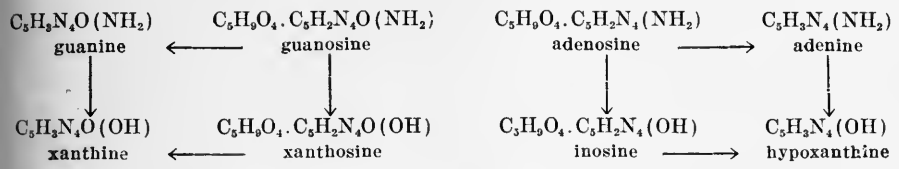
The chemistry of the nucleosides is just what one would suppose *a priori,* and it follows closely that of the simple nitrogen derivatives. They offer two possibilities, (1) hydrolysis, (2) deaminization. Thus by hydrolysis, adenosine and guanosine are decomposed into pentose and the respective purine bases.

$$\underset{\text{adenosine}}{C_5H_9O_4\,.\,C_5H_4N_5} + H_2O = \underset{\text{pentose}}{C_5H_{10}O_5} + \underset{\text{adenine}}{C_5H_5N_5}$$

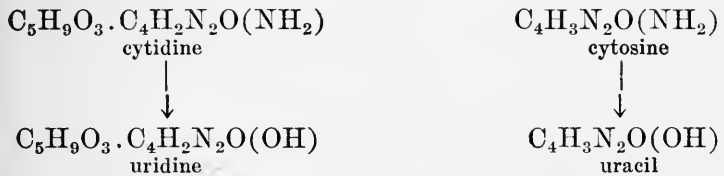
$$\underset{\text{guanosine}}{C_5H_9O_4\,.\,C_5H_4N_5O} + H_2O = \underset{\text{pentose}}{C_5H_{10}O_5} + \underset{\text{guanine}}{C_5H_5N_5O}$$

Just as the free amino-purines (guanine and adenine) are deaminized to the corresponding oxy-purines (xanthine and hypoxanthine), so also the amino-nucleosides (guanosine and adenosine) form the corresponding oxy-nucleosides (xanthosine and inosine).

These relations are shown in the following diagram. Horizontal arrows indicate hydrolysis; vertical arrows, deaminization.



With the pyrimidine nucleosides the matter is a little different. Deaminization converts the amino-nucleoside (cytidine) into its corresponding oxy-nucleoside (uridine).

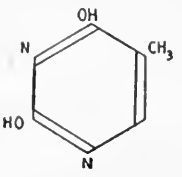


But the two pyrimidine nucleosides are very stable, and are not hydrolyzed by mineral acid into pentose and free pyrimidine as is the case with the purine nucleosides. Of course it is possible that animal ferments are capable of effecting hydrolysis of the pyrimidine nucleosides.

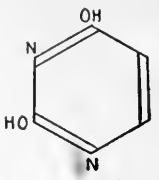
One might therefore suspect that the metabolism of yeast nucleic acid is a play upon hydrolysis, deaminization and oxidation, which will produce various nucleotides, nucleosides and free bases, and if continued far enough must finally end in the formation of uric acid. In Part II it will be shown that such is actually the case.

ANIMAL NUCLEIC ACID

The chemistry of thymus nucleic acid is best appreciated by a comparison of the substance with yeast nucleic acid. When thymus nucleic acid is boiled with dilute sulphuric acid it easily sets free both of the amino-purines (guanine and adenine), with part of its phosphoric acid and part of its carbohydrate. But when thymus nucleic acid is submitted to severe acid hydrolysis (as with 30 per cent sulphuric acid at 150°), the two pyrimidine derivatives are set free with the remainder of the carbohydrate and phosphoric acid. All of these statements are equally true for yeast nucleic acid; but it must be noted that thymus nucleic acid yields thymine (Kossel and Neuman (*a*)(*b*), (1893, 1894)) where yeast nucleic acid yields uracil.



thymine



uracil

Another point of difference between the two nucleic acids is in respect to their carbohydrate group. The carbohydrate group of yeast nucleic acid is a pentose group, and a pentose is formed by hydrolysis of the nucleic acid; but the carbohydrate group of thymus nucleic acid is a hexose group, and the *decomposition products* of a hexose (formic acid and levulinic acid) are formed by hydrolysis of the nucleic acid.

$$C_6H_{12}O_6 = \underset{\text{levulinic acid}}{CH_3CO.CH_2CO_2H} + HCO_2H$$

The fundamental groups of the two nucleic acids are therefore as follows

	Of Thymus Nucleic Acid	*Of Yeast Nucleic Acid*
1.	Phosphoric acid	Phosphoric Acid
	Purine Derivatives	
2.	Guanine	Guanine
3.	Adenine	Adenine
	Pyrimidine Derivatives	
4.	Cytosine	Cytosine
5.	Thymine	Uracil
	Carbohydrate	
6.	Hexose	Pentose

This fundamental identity or analogy of the two nucleic acids is very striking, especially in connection with their curious and parallel hydrolytic conduct; and it strongly suggests that the two nucleic acids have a similar chemical constitution. Such a question, however, can only be decided by a study of the partial decomposition products of thymus nucleic acid, and in such a study one must be careful lest he fall into the "argument in a circle." Thus, the constitution of thymus nucleic acid may be assumed in the beginning, and from this assumed constitution, that of its decomposition products may be inferred. The latter may then be used to prove the constitution of the nucleic acid. The matter is mentioned here, not in disparagement of the work that has been done with the products of the partial hydrolysis of thymus nucleic acid, but because the writer believes that the logical fallacy indicated has occurred in the original discussion of the subject.

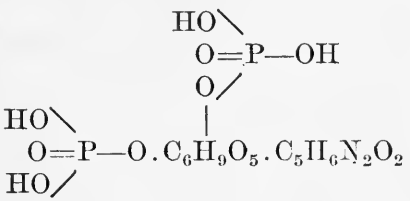
THE PARTIAL DECOMPOSITION PRODUCTS OF THYMUS NUCLEIC ACID

Levene and Mandel (*a*) (1908) prepared an indefinite substance from thymus nucleic acid which produced phosphoric acid, levulinic acid and thymine. They conclude that the substance is thymine-hexa-nucleotide.

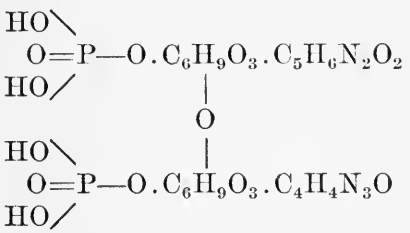
Levene and Jacobs (*i*) (1913) prepared a substance from thymus nucleic acid that forms guanine and levulinic acid. It is possibly guanine-hexa-nucleoside.

If these two substances, one a nucleoside and the other a nucleotide, indicate that thymus nucleic acid is constructed throughout upon nucleosides and nucleotides, then the later work of Levene and Jacobs (*j*) (1912) suggests the structure of thymus nucleic acid. Their argument is based upon the assumed structures of three compounds which they obtained by the mild hydrolysis of thymus nucleic acid with sulphuric acid.

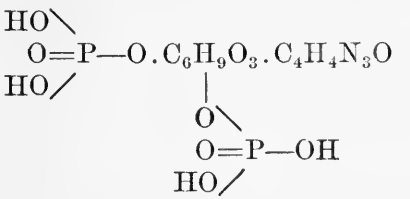
1. Hexa-thymidine di-phosphoric acid
2. Hexa-cytidine di-phosphoric acid
3. Hexa-cytosine-thymine-di-nucleotide[1]



Thymidine Di-phosphoric Acid



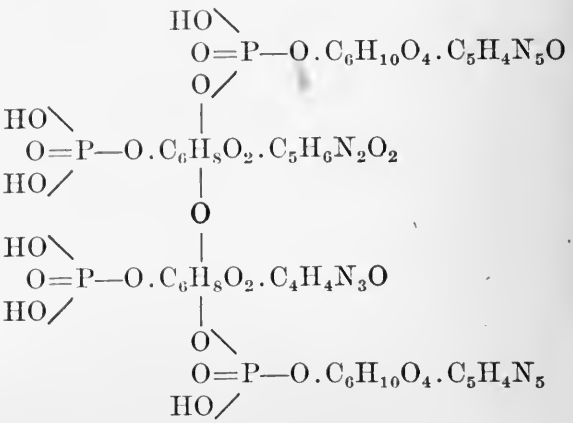
Thymine-Cytosine Di-nucleotide



Cytidine Di-phosphoric Acid

[1] In the nomenclature of the decomposition products of nucleic acids the prefixes "penta" and "hexa" have reference to the carbohydrate groups. "Hexa" means "from thymus nucleic acid"; "penta" means "from yeast nucleic acid."

If the structures of these compounds be admitted, then the constitution of thymus nucleic acid is indicated.



Reduced to its simplest terms, this complicated formula means the following:

1. Thymus nucleic acid, like yeast nucleic acid, is a tetra-nucleotide composed of the groups of four mono-nucleotides.

2. The linkages that join the four mono-nucleotide groups to one another are differently located in the two nucleic acids.

With the latter statement physiology is at present little concerned. With the former statement physiology is very much concerned; for the decomposition of the two nucleic acids under the influence of animal ferments follows parallel lines. With reference to animal metabolism the two nucleic acids have an "equivalent" structure.[2]

Physiological Part

THE PHYSIOLOGICAL DECOMPOSITION OF NUCLEIC ACID

The discovery of nucleic acid in the tissues naturally prompted a host of investigations to find a physiological agent capable of decomposing the substance. It was assumed, without justification, that such a decomposition would involve the simultaneous disruption of all of its linkages with the simultaneous production of all of its fundamental decomposition products. Of these substances, only phosphoric acid and the purine bases can

[2] While this article was in press Levene abandoned the above formula for thymus nucleic acid (J. Biol. Chem., 48, 1921, 122) and Thannhauser has added an important contribution to the subject. (Thannhauser and Ottenstein, Zeits. f. physiol. Chem., 114, 1921, 39.)

be easily detected, and as free phosphoric acid is constantly present in tissue extracts, the decomposition of nucleic acid was generally considered proven, when a free purine base appeared during the digestion of material at the body temperature.

All of the earlier work upon this subject was confused by unavoidable sources of error. The physiological decomposition of nucleic acid could not be clearly followed until after the chemistry of the substance had reached a comprehensive stage. Methods of isolating and separating the decomposition products were not known; in fact, the identity of the purine bases themselves was not established until very late. Chemists were limited to one decomposition product, and to one reagent for its detection. Putrefaction played an important part that was not taken into account.

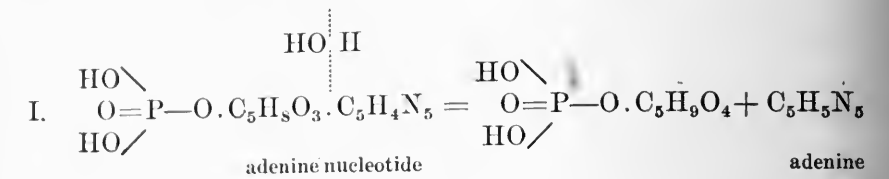
These are a few of the many circumstances that not only put the earlier investigators at a great disadvantage, but made their work difficult to understand and in some cases impossible to interpret. It is, therefore, not in derogation of many of these obscure investigations, but in the interest of clearness that we pass immediately to the work of Iwanoff (1903).

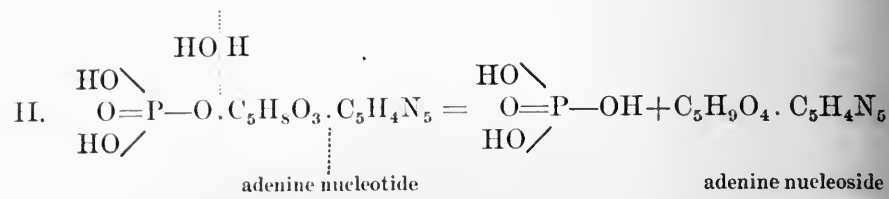
He cultivated various molds (Penicillium glaucum and Aspergillus niger) on thymus nucleic acid, and found that both phosphoric acid and purine bases were produced as the molds grew, although there was not present any ferment that could hydrolyze a protein. Iwanoff naturally concluded that he was dealing with a specific ferment, adapted to the decomposition of nucleic acid, and called it "nuclease." Shortly, following this work, many researches were reported to show the existence of a similar ferment in animal and plant tissues, so that the wide distribution of nuclease was soon conceded.

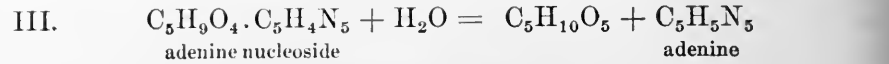
But it was shown later that the physiological decomposition of nucleic acid is a rather complicated matter involving a number of active agents, and that various gland extracts differ markedly from one another in the extent to which they can carry this decomposition. It is certain that the first stage consists in the disruption of the nucleotide linkages with the consequent production of simpler nucleotides, but without setting free either phosphoric acid or purine bases. (Jones (*e*), 1920.) It would be proper to apply the term nuclease to this ferment, or to abandon the term altogether, since it can have no such meaning as was originally ascribed to it.

Leaving out of consideration the two pyrimidine nucleotides (of which little is known), the purine nucleotides may undergo enzymatic decomposition in either of two ways, depending on the particular physiological agent that they encounter. The purine base may be set free, or the phosphoric acid may be liberated with the production of a nucleoside.

Finally, the nucleosides under proper enzymatic conditions decompose into free purine and carbohydrate.







Purine bases are, therefore, produced in the nuclein metabolism along different lines, and their subsequent conversion into uric also occurs along different lines. The intention of the following pages is a discussion of these various paths from nucleic acid to uric acid, and it would be logical to proceed from nucleic acid, but it is more convenient to begin at the end, and end at the beginning.

The Formation of Uric Acid from Nucleic Acid.—Uric acid was formerly supposed to be an intermediate product in protein metabolism, but its specific origin was clearly indicated when the purine groups of nucleic acid were discovered; and endeavors were naturally made to place this indication on an experimental basis. Horbaczewski (*b*)(*c*) (1889, 1891) was the first to do this. His results are fundamental and quickly told. Calf's spleen was ground to a pulp with water, and kept at the body temperature until putrefaction was well advanced. The putrid product was then sterilized by the addition of lead acetate, arterial blood was added, and the material was allowed to digest at 40° as a slow stream of air was passed. In the end, uric acid could be found, while similar experiments in which no air was passed produced xanthine and hypoxanthine instead of uric acid.

Horbaczewski did not clearly understand what he was doing and took a great deal of useless trouble. The preliminary putrefaction and the use of arterial blood were superfluous procedures while the sterilization with lead acetate might have vitiated his results. Nevertheless, he started with nucleic acid of spleen pulp and ended with uric acid.

Horbaczewski also found that in man the ingestion of nucleic acid pro-

duced an increase of uric acid in the urine, whereupon he formulated the well known leucocytosis theory.

It is frequently stated that the entire work of Horbaczewski was "unintelligent"; yet he showed the physiological origin of uric acid from nucleic acid, and thus solved one of the most important physiological problems of his day.

The Formation of Uric Acid from the Oxy-purins.—Of Horbaczewski's many vagaries, perhaps the most serious was his misconception of the path along which uric acid is formed from nucleic acid. He stated positively, that as no one had been able to oxidize either xanthine or hypoxanthine to uric acid outside of the body, these substances could not be intermediate products in the passage from nucleic acid to uric acid, and therefore, the purine groups of nucleic acid must have been deaminized and oxidized before they were set free. However this may be, Spitzer (1899) found that an aqueous extract of spleen can bring about the required oxidation. To the extract he added a weighed amount of oxy-purine and digested the mixture at 40°, as a slow current of air was passed. The oxy-purine disappeared and in its place was found a reasonable equivalent of uric acid. The active agent that brings about the transformation is called xanthine-oxidase. Its presence can be shown in tissue extracts that are devoid of power to bring about other purine transformations; hence xanthine-oxidase is specific.

The Formation of Oxy-purines from Amino-purines.—In order to pass from nucleic acid to uric acid three transformations are required (though not necessarily in the order given).

1. Liberation of the purines
2. Deaminization
3. Oxidation

Of these three, deaminization remains to be considered.

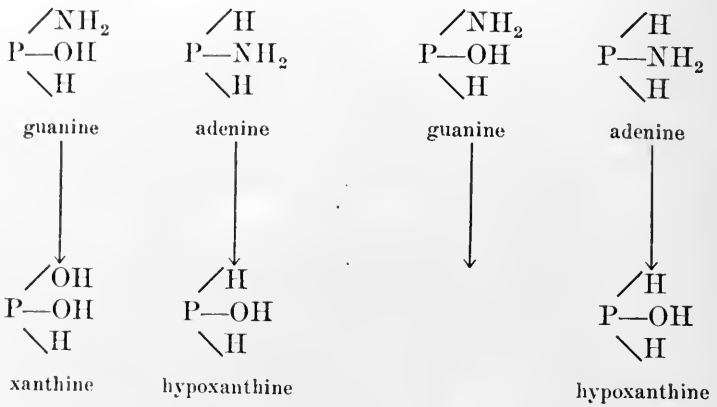
All gland extracts contain nucleic acid; so that the purine ferments may be studied by examining the purine products of autodigestion. When an aqueous extract of pig's pancreas is allowed to digest at 40°, free purine bases soon make their appearance. They are not, however, the amino-purines (guanine and adenine) that one would expect to be formed from nucleic acid, but the two corresponding oxy-purines (xanthine and hypoxanthine). The same results are obtained with thymus. These experiments lead to the assumption that in the digestion, the amino-purines are first formed but are subsequently converted into the oxy-purines by a deaminizing agent present in the tissue extract.

A most unexpected result was obtained with pig's spleen. The end products of the self-digestion of an aqueous extract of this tissue are guanine and hypoxanthine, *i.e.*, one amino-purine, and one oxy-purine. It is reasonable to suppose that initially both amino-purines are liberated from

the nucleic acid of the gland extract, but only one of them is subsequently deaminized. This necessitates the conclusion that both thymus and pancreas contain two independent deaminizing ferments (guanase and adenase), only one of which (adenase) is present in the spleen.

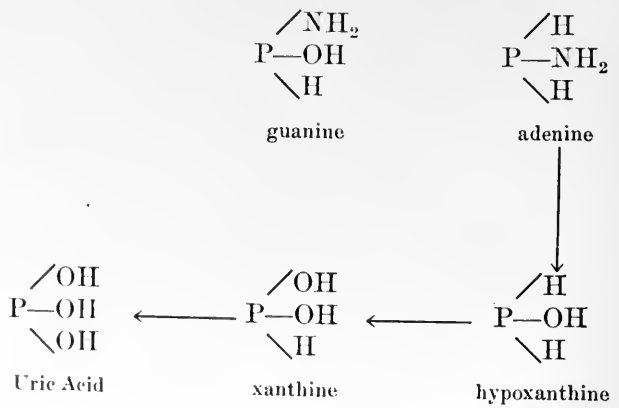
An equally curious result was obtained with pig's liver. The end products of self-digestion are guanine and xanthine. This is easily accounted for by assuming that the guanine set free from the nucleic acid remains unchanged, but that the adenine is deaminized to hypoxanthine, which in turn is oxidized to xanthine.

Representing the purine ring with its three replaceable hydrogen atoms by the symbol $P{-}H_3$, the results of autodigestion may be expressed as follows:



Pig's Thymus and Pancreas (Jones and Austrian (*a*), 1906)

Pig's Spleen (Jones and Winternitz, 1905).



Pig's Liver (Jones and Winternitz, 1905)

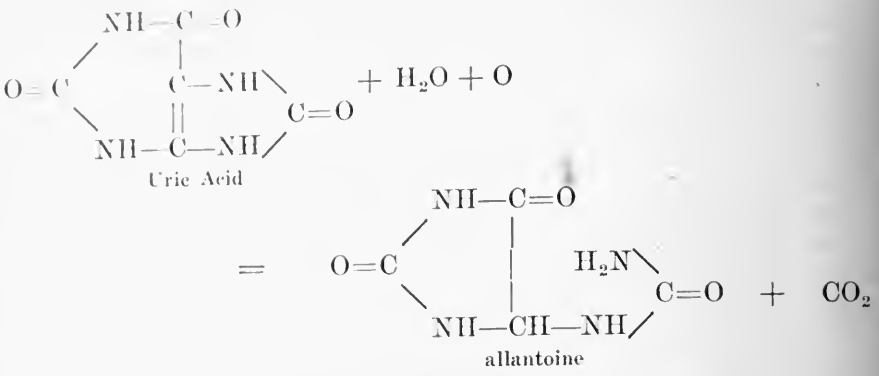
But these considerations are somewhat speculative. There is but one way to prove the presence of a ferment. The substance supposed to be decomposed must be introduced; as digestion proceeds it must disappear, and in its place must be found a reasonable equivalent of the substance supposed to be formed. Accordingly, dilute aqueous extracts of the various tissues were prepared and portions taken so small that the purine bases formed from the extract itself could be ignored. The purine base in question was then added to the tissue extract, the material was allowed to digest at 40° under antiseptic conditions, and the product was finally examined for purine bases. In this way each of the glands was found to possess the ferments that had been indicated by the results of autodigestion. Thymus converted guanine into xanthine, and adenine into hypoxanthine. Pancreas did the same. Spleen converted adenine into hypoxanthine, but left guanine unchanged. Liver converted adenine into hypoxanthine, and hypoxanthine into xanthine, but left guanine unchanged. Three independent factors of purine fermentation are thus disclosed (Jones (*a*), 1905).

1. *guanase,* 2. *adenase,* 3. *xanthine oxidase*

Dog's liver contains guanase but not adenase; pig's spleen contains adenase but not guanase; neither tissue contains xanthine-oxidase. The three ferments are therefore independent of one another.

THE PHYSIOLOGICAL DESTRUCTION OF URIC ACID

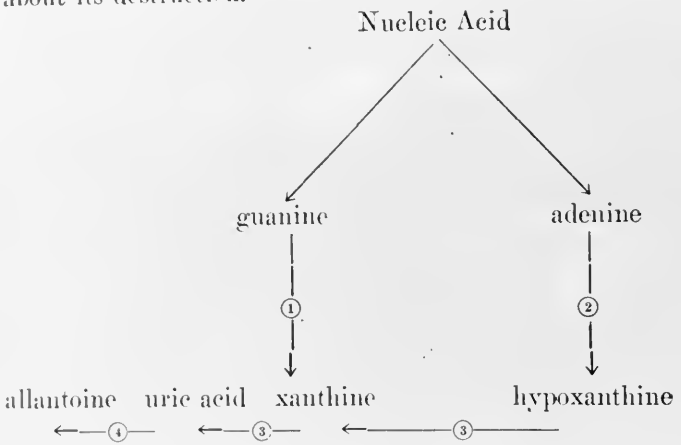
Many experimenters have observed that uric acid may be made to disappear by digestion at 40° with aqueous extracts of certain glands in the presence of a sufficient supply of oxygen. But the disappearance of uric acid and its physiological destruction are two different things. While undoubtedly an element of truth permeated all of the earlier work, this work is so full of error and confusion that we must look upon much of it as a fortunate accident. Uric acid was destroyed by laboratory methods used in examining the products of digestion, or was lost in coagula. Its destruction product was incorrectly stated to be glycocoll, oxalic acid or nothing at all. So that even now a considerable amount of ingenuity is required to value the results of the early workers. A great deal of time can be saved and annoyance avoided by proceeding directly to the modern well-established conclusion that certain tissue extracts are capable of bringing about the conversion of uric acid into the more soluble allantoine provided that a sufficient amount of air be supplied. (Wiechowski (*a*) (*b*) (*c*) (*d*).) The gradual emergence of this truth from a mass of obstructing error is most interesting. While the principal credit is given to Wiechowski, it is difficult to say who really made the discovery.



Thus the purine fermentation is effected by four independent physiological agents.

1. *guanase,* 2. *adenase,* 3. *xanthine-oxidase,* 4. *uricase.*

Three of these lead up to the formation of uric acid and the fourth brings about its destruction.

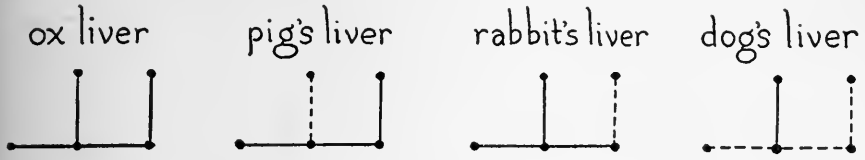


A study of the localization of these ferments discloses interesting and important matter.

THE DISTRIBUTION OF THE PURINE FERMENTS

1. With very rare exceptions, the four ferments of the purine fermentation are not present in any one tissue. The distribution characterizes the tissue and the species. This variation of the distribution with species, as well as the independent existence of guanase, adenase and xanthine-oxidase is shown by an examination of the livers of four different species. (Jones and Austrian (*a*) (1906).) Ox liver forms uric acid from both amino-purines, pig's liver from only one (adenine), rabbit's liver only

from the other (guanine), and dog's liver from neither. The results are shown in the following diagrams which are abbreviations of the one on page 138. The absence of a ferment is indicated by a dotted line.



2. The purine ferments do not appear in an organ simultaneously, but are formed successively as embryonic development proceeds; so that the distribution depends not only upon the particular tissue and the species, but to a considerable extent upon the age of the animal. None of the purine ferments can be demonstrated in the aqueous liver extract of a pig embryo less than 90 mm. in length. As the embryo increases in length from 90 mm. to 200 mm., adenase makes its appearance, but xanthine oxidase appears only after the birth of the animal. (Jones and Austrian 1907.)

3. The distribution of the purine ferments in the organs of man is very characteristic. Adenase is not present in any human tissue. Guanase is irregularly distributed, being present in the kidney, liver and lung but absent from the spleen and pancreas. (Jones and Austrian (*b*).) It is significant that human urine contains adenine, but not guanine. Xanthine oxidase is profusely present in the human liver but is confined to the one organ. (Miller and Jones; Winternitz and Jones.)

Uricase is not present in the liver, nor in any other organ either of children or adults, nor is allantoine present in human urine, except a trace of the substance that is ingested with the food. It seems curious that man should have lost so useful a function as ability to destroy uric acid.

4. Uricase may be regarded as a liver ferment since it is probably present in the livers of all the lower animals except the ape (ox, dog, pig, sheep, rabbit, guinea pig, horse, rat, opossum, monkey), and except for an occasional occurrence in the spleen (ox), the ferment is found only in the liver. Its location makes it very effective, so that allantoine is far more abundant than uric acid in the urine of the lower animals. This appears in the analyses of the urine of seventeen animals, twelve of which were made by Hunter and his associates. They calculate a factor for each animal species called the "uricolytic index," which is directly proportional to the allantoine, and inversely proportional to the uric acid. The following table, adapted from that of Hunter and Givens (*c*) (1914), shows the great preponderance of the allantoine over the uric acid in the urine of the lower animals, in contrast to the urine of man and the ape.

Animal Species.	Uricolytic Index.
Opposum	79
Sheep	80
Horse	88
Monkey	89
Goat	92
Cow	93
Guinea pig	94
Rabbit	95
Raccoon	95
Rat	96
Coyote	97
Cat	97
Dog	98
Badger	98
Pig	98
Ape	0
Man	0

5. Xanthine oxidase, like uricase, is generally confined to the liver (ox, pig, rabbit, guinea pig, opossum, man), but is not so widely distributed as uricase. Thus certain livers (rat and dog) are provided with a ferment to destroy uric acid but with none to form it. This is not an uncommon circumstance. Rabbit's liver is able to oxidize hypoxanthine to uric acid, but cannot form hypoxanthine from adenine.

Perhaps the most active occurrence of xanthine oxidase is in human liver, which accords with man's low output of purine bases, the ratio of purine bases to uric acid being thirty-five times greater in monkey's urine than in human urine.

The deficiency of xanthine oxidase in the organism of the monkey (cercopithecus) was noted by Hunter. In a haphazard quantity of urine he found

Uric acid	.320
Xanthine	.950
Hypoxanthine	.360
Guanine	.000
Adenine	.000

Even subcutaneously injected xanthine was recovered unchanged. (Hunter and Givens (*b*).)

Xanthine oxidase is not present in yeast where such a multitude of ferments occur, nor is uric acid to be found in plants.

6. Guanase is the most widely distributed of all the purine ferments. With many animal species it is uniformly present in all of the principal organs (rat, ox, guinea pig, rabbit). But pig's organs are peculiarly deficient in the ferment, and the muscles of the animal frequently contain deposits of guanine, due perhaps to "guanine gout." (Virchow (*a*) (*b*), 1866, 1866.) Pig's urine contains guanine and the purine bases are always in excess of the uric acid. (Pecile; Mendel and Lyman.)

7. Adenase, on the contrary, is very rare, having a distribution that is somewhat complementary to that of guanase. Its presence cannot be shown in any of the principal organs of the rat, man or rabbit. As the two

ferments are seldom associated with one another, it seems queer that they should ever have been thought identical.

Muscular hypoxanthine, which forms a considerable part of what Burian and Schur call "endogenous" uric acid, is not the result of the action of adenase on adenine. Leonard and Jones were not able to observe a transformation of adenine into hypoxanthine by aqueous extracts of muscle, while Voegtlin and Jones found that perfused adenine is not altered by surviving muscle.

But the path of adenine metabolism does not always pass through hypoxanthine. None of the organs of the rat exhibit adenase (Rohde and Jones), and Nicolaier found that in rats subcutaneously injected adenine is oxidized but reaches the kidney without deaminization where it forms concretions of 6-amino-2-8-dioxypurine.

$$\begin{array}{ccc} \diagup H & & \diagup OH \\ P{-}NH_2 + 2O & = & P{-}NH_2 \\ \diagdown H & & \diagdown OH \\ \text{adenine} & & \text{6-amino-2-8-dioxy-purine} \end{array}$$

Ebstein and Bendix found a similar transformation of adenine in the organism of the rabbit. But these two are the only authentic cases in the literature where oxidation of a free amino-purine was found to occur without deaminization.

8. The distribution of the purine ferments is often obscure, because a given tissue extract may be able to bring about the decomposition of a combined purine but unable to effect a similar decomposition of the free base. Thus, dog's liver cannot convert free adenine into hypoxanthine, but it can form hypoxanthine from nucleic acid with the greatest ease. Human tissues do not contain adenase, yet the subcutaneous injection of adenosine causes a marked increase of uric acid. (Thannhauser and Bommes.)

A purine base may even undergo both deaminization and oxidation while still combined. Benedict (*a*) (1915) has shown that about 90 per cent of the uric acid of ox blood is in combined form. It is present only in the corpuscle and is set free by a ferment present when the blood is allowed to stand. This contrasts sharply with the uric acid of chickens' blood, which does not have a purine precursor. Here the uric acid is all free and in the plasma.

Bass found that the purine bases of human blood are combined, and can only be detected after acid hydrolysis. He was able to isolate adenine but at most only traces of guanine.

9. The purine metabolism does not always suggest evolutionary relations, but it often does. The proof that uricase is not present in the tissue extracts of either the ape or man, and that allantoine is not present in the urine of either species (Wiechowski (*e*)), surely justifies all the

labor that has been expended upon the purine metabolism. Both species also fail to exhibit adenase, and exhibit guanase irregularly in the various organs. (Wells and Caldwell.)

The gradation from man to ape to monkey in relation to adenase is interesting. Hunter and Givens (*b*) found that injected adenine was largely excreted unchanged in the urine of the monkey *Cercopithecus,* and Hunter and Givens (*a*) were able to show adenase in slight activity in organ extracts of a second monkey *Cebus apella.* With organ extracts of a third monkey *Macacus rhesus,* Wells was able to obtain a striking demonstration of adenase.

The distributions of the purine ferments in the organs of the rabbit and guinea pig are coincident throughout. (Mitchell.)

10. The purine metabolism of the rat is curious. Rohdé and Jones found that neither the individual organs nor the combined organs of the rat exhibit xanthine oxidase in spite of the fact that they could show the plentiful presence of uric acid in rat's urine. They also found that the combined organs of the rat could not change hypoxanthine. This apparent contradiction is not different from many similar cases, and could be accounted for by assuming that in rats, uric acid is formed along a path that does not involve xanthine-oxidase. But Ackroyd (*b*) found that the injection of hypoxanthine causes an increase in the allantoine of rat's urine. This was a most puzzling matter until the work of Benedict (*b*) appeared.

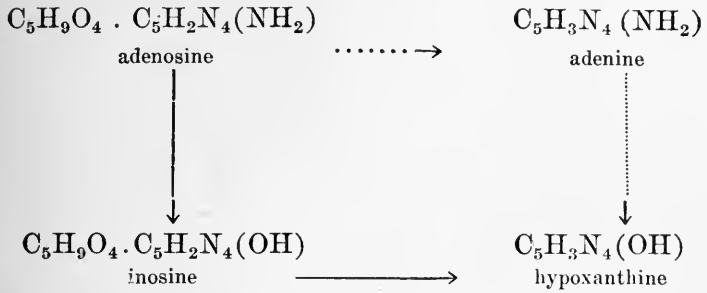
11. Benedict found that the Dalmatian coach hound excretes both allantoine and uric acid, and that when the urine of the animal is acidified with hydrochloric acid, a crystalline deposit of uric acid is formed. Careful analyses of the dog's urine were made for both allantoine and uric acid, over a long period of time, and then uric acid was injected subcutaneously. This caused the expected rise in the allantoine but the injected uric acid also appeared, and quantitatively. From these results Benedict concludes that "uric acid and allantoine are interrelated in metabolism in other ways than have heretofore been assumed."

THE ENZYMATIC DECOMPOSITION OF COMBINED PURINS

Many observations indicate that the organism treats combined purines differently from free purines. The following two experiments go to the root of the matter.

I. When adenine is digested for several days with an aqueous extract of dog's liver, the substance remains unaltered and can be recovered. Dog's liver does not contain adenase. But when nucleic acid (yeast or thymus) is digested with an aqueous extract of dog's liver, hypoxanthine is formed in an amount corresponding to the adenine group of the nucleic acid used. This is very clear. Dog's liver can deaminize combine adenine,

but not free adenine. The tissue contains both adenosine deaminase and inosine hydrolase but neither adenosine hydrolase nor adenase (Amberg and Jones), as indicated in the diagram:



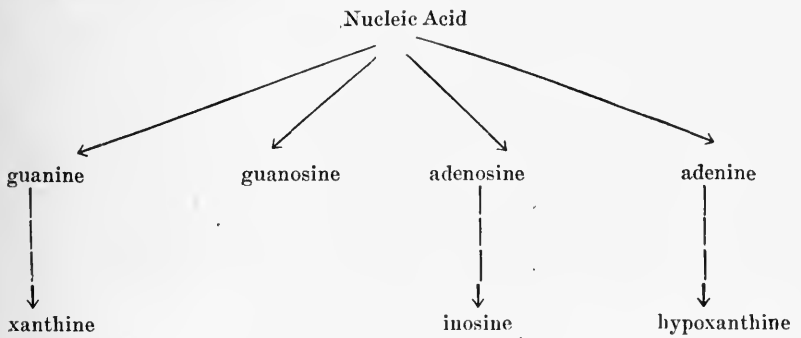
In the nuclein metabolism there are two paths to hypoxanthine, one of which cannot be used by dog's liver.

II. When an aqueous extract of pig's pancreas is allowed to digest at 40° C., xanthine and hypoxanthine are formed. This was to be expected because the gland contains both guanase and adenase. But when the digested extract is boiled with dilute mineral acid the free purines are greatly increased. Guanine and additional hypoxanthine appear.

These results can be explained in only one way. The nucleic acid is first decomposed into its simple nucleotides, as was to be expected. Each of the purine nucleotides is then decomposed in two ways by the action of two ferments present in the gland extract. In one way, the purine base is set free (action of purine nuclease), and in the other way, phosphoric acid is split off leaving the nucleoside (phospho-nuclease). Thus in the self-digestion of the pancreas four purine compounds are initially produced; guanine, adenine, guanine nucleoside, adenine nucleoside.

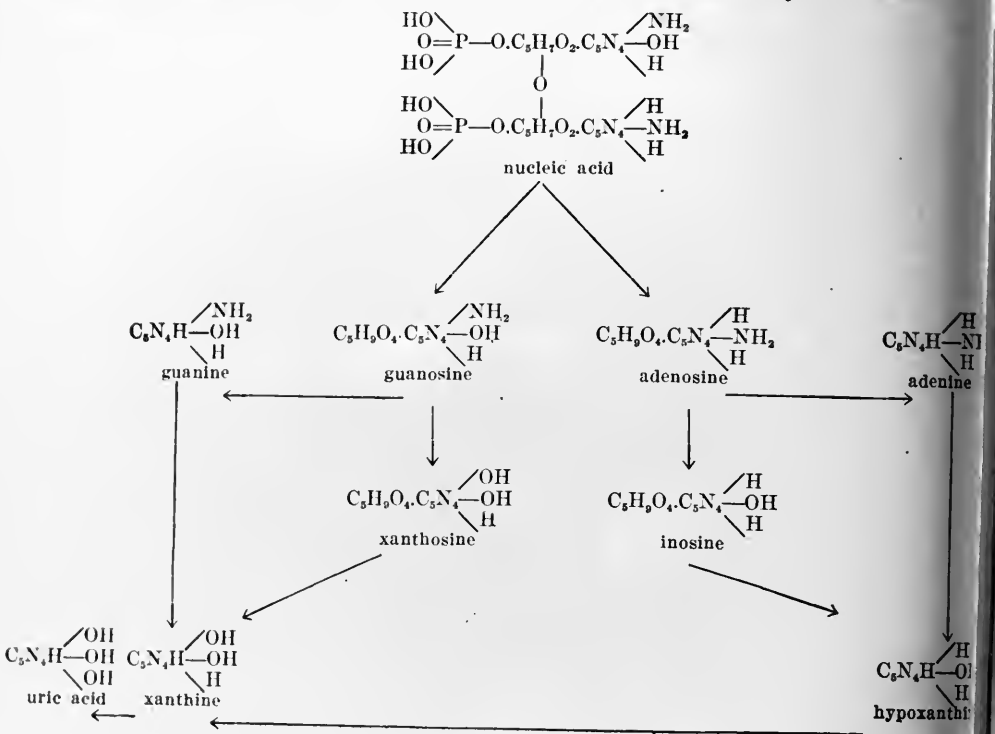
The two free purines are deaminized and we therefore find the oxypurines among the products. The adenine nucleoside is also deaminized to hypoxanthine nucleoside but the guanine nucleoside is not similarly deaminized. Hence subsequent acid hydrolysis produces guanine and hypoxanthine.

Using the terminology of yeast nucleic acid, the autolysis of pig's pancreas is expressed in the following diagram



The gland evidently contains adenase, guanase and adenosine deaminase, but not guanosine deaminase (Jones (*b*) 1911.)

By similar experiments and similar reasoning the localization of the nucleic ferments of many glands has been shown but much space would be required to consider the individual cases. The general scheme of nuclein metabolism so far as it concerns purine derivatives, is indicated in the following diagram which shows the theoretical possibilities, nucleic acid being represented as a di-purine di-nucleotide. The independent existence of each ferment indicated by an arrow has been fairly well shown.



Urobilin and Urobilinogen

LOUIS BAUMAN

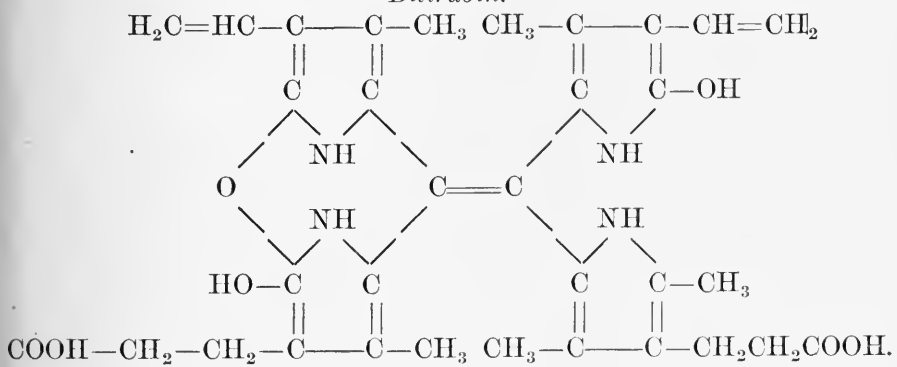
NEW YORK

Chemistry

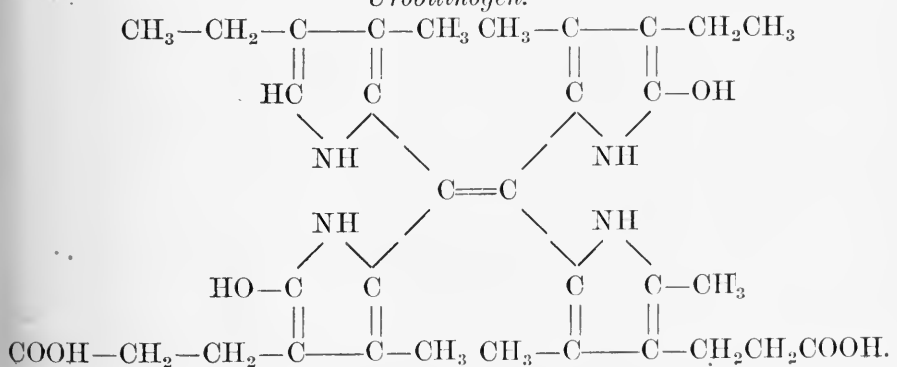
In 1868 Jaffe first described a reddish substance which he found in human and canine bile and which resembled one of the urinary pigments. Both absorbed certain rays between the B and F lines of the spectrum and both fluoresced in the presence of zinc salts. Jaffe named the compound urobilin. It is interesting to note that even at that time he was aware that the pigment was not preformed, but resulted from the oxidation of a chromogen, which is now known as urobilinogen (LeNobel).

Urobilinogen has the empirical formula, $C_{33}H_{42}O_6N_4$. Fischer and Roese showed that it contained 4 pyrole nuclei and that its structural formula closely resembled that of bilirubin.

Bilirubin.



Urobilinogen.



Urobilinogen is a colorless compound which forms monoclinic crystals melting at 192° C. Its molecular weight is 600. It is soluble in chloroform and other organic solvents and is readily oxidized to urobilin by the oxygen of the air and by oxidizing substances.

H. Fischer synthesized urobilinogen by reducing bilirubin with sodium amalgam; he also described some of its physical and chemical properties. He obtained it to the extent of about 46 per cent of the bilirubin which he employed, and assuming that it was derived from one-half of the bilirubin molecule he named it hemibilirubin. Later Fischer and Meyer-Betz (*a*) (1911) proved that urobilinogen and hemibilirubin were identical. Fromholdt obtained the same substance by a somewhat similar method.

When urobilinogen is treated with para-dimethylamino-benzaldehyd, dissolved in hydrochloric acid, the so-called Ehrlich reagent, it forms a red compound which absorbs certain rays in the orange and green regions of the spectrum between the D and E lines. The red compound results from the oxidation of a colorless chromogen. A solution containing one part of urobilinogen in 640,000 parts of water still gives the Ehrlich reaction (Fischer and Meyer-Betz (*a*), 1911). This reaction is not specific, for it is obtained with any pyrole derivative that contains a free hydrogen atom attached to one of the carbon atoms of the ring. Urine containing indol derivatives also gives the color test but does not exhibit the characteristic absorption bands (Fischer).

Urobilin is easily obtained from urobilinogen by oxidation. It is a reddish yellow or brown substance of uncertain composition, and probably contains a number of urobilinogen molecules that have been oxidized and polymerized. It is soluble in aqueous alkali and in most organic solvents such as alcohol, ether and chloroform. Urobilin absorbs certain rays in the region of the B and F lines of the spectrum. It forms a colored salt with mercuric chlorid, the so-called Schmidt test. When an alkaline solution of urobilin is neutralized with copper sulphate solution a red compound, soluble in chloroform, is formed. This copper compound exhibits the characteristic urobilin absorption bands (Bogomolow). Urobilin is precipitated from watery solution by ammonium sulphate. It can be reduced to urobilinogen by bacteria (Charnas). Fischer isolated 160 grams of urobilin from a large amount of human feces. His analysis, carbon 63.46 per cent, hydrogen 7.67 per cent, and nitrogen 4.09 per cent, agreed with that reported by Garrod and Hopkins about 14 years previously. When urobilin was subjected to dry distillation or reduction by glacial acetic acid and zinc dust two substances were obtained. The one contained nitrogen while the other resembled cholesterol or one of the bile acids, and did not contain nitrogen.

Occurrence.—Because urobilin and urobilinogen have the same clinical and physiological significance, and for the sake of brevity, the term urobilin will be used to include both substances.

Urobilin occurs in normal bile and in normal stool except in that of the new-born. It is present in the urine in negligible quantities. Concerning its presence in the blood there is little definite information. If it occurs therein it is not demonstrable by our present methods. The writer has frequently attempted to determine its presence in the serum of patients that were excreting considerable quantities in the urine and stool, but without avail. When normal serum is heated with strong hydrochloric acid a positive Ehrlich reaction is obtained, but this is probably due to decomposition of one of the heterocyclic amino acids, such as tryptophan. Gerhardt and others have obtained the reaction with serous fluids other than blood. Conner and Roper claim to have found it in the serum of pneumonia patients shortly before death. When urobilin is added to blood it rapidly disappears probably as a result of oxidation by oxyhemoglobin (Roth and Herzfeld).

An increased amount of urobilin is found in the stool, in the bile, and occasionally in the urine, in pernicious anemia and other conditions associated with a destruction of red blood cells, and also in diffuse lesions of the liver. Urobilin is absent from the stool in jaundice due to complete closure of the common bile duct and in severe diarrhea.

Mechanism of Urobilin Formation.—The voluminous literature pertaining to this subject abounds in theoretic discussion and hypotheses. The enterogenous theory had its chief exponent in Friederich Mueller (*b*) (1892). It appears to be least open to criticism, and is supported by numerous clinical and experimental observations. It postulates that urobilin results from the reduction of bilirubin by the bacteria of the large intestine. The following evidence is submitted in support of the enterogenous theory: 1. The transformation of bilirubin into urobilin in vitro by bacteria (Mueller, 1892 (*a*); Fischler (*a*), 1906). 2. Urobilin is absent from the stool and urine of severely jaundiced patients but appears when urobilin-free bile is administered by stomach tube (F. Mueller (*b*), 1892). 3. Bilirubin alone is found in the intestine of the new-born until the third day, when urobilin appears coincident with the development of the bacterial flora. 4. Diarrheal stools often contain bilirubin but no urobilin. This is apparently due to the rapid propulsion of the intestinal contents —that is, the stool is expelled before the bacteria have had an opportunity to reduce bilirubin. 5. Urobilin is not present in the small intestine where bacteria are absent, but appears distally to the ileocecal valve (Schmidt).

Normally some urobilin is absorbed from the large intestine and brought to the liver where it is partly excreted into the bile and partly converted into another substance, probably bilirubin. The liver does not permit urobilin to escape into the general circulation. The traces that are normally found in the urine may be due to absorption from the lower bowel by the blood of the inferior hemorrhoidal plexus.

When extensively diseased the liver may permit urobilin to escape into

the general circulation and then it is excreted by the kidneys. In conditions causing a rapid disintegration of red blood cells, as in pernicious anemia, hemolytic jaundice, internal hemorrhage, etc., a large amount of hematin is converted into bilirubin, and this permits an increased absorption of urobilin from the intestine. Under these circumstances some urobilin may escape into the general circulation even though the liver be functionally intact. In recent years hematin and bilirubin have been demonstrated in the blood serum in pernicious anemia (Schumm).

While the enterogenous theory explains most of the known facts it does not satisfactorily account for all of the experimental results recorded in the literature. Fischler (*a*) (*b*) (1906, 1908) has submitted evidence favoring the liver itself as a site of urobilin formation. The following experiments may be cited in this connection: When the common bile duct of dogs is tied and a biliary fistula is established it is found that in spite of the deviation of the bile to the exterior urobilin persists in the stool but disappears from the bile. If, to such animals, poisons that exert a particularly destructive effect on the liver parenchyma such as ethyl alcohol, amyl alcohol and phosphorus, be administered there results a large increase in the urobilin content of the bile and a lesser increase in the feces. Fischler maintains that under these conditions the liver itself produces urobilin some of which is absorbed by the blood and excreted into the intestine. The disturbing features in Fischler's experiments were the lack of uniform results, the licking up of bile from the fistula by some of the dogs and the presence of jaundice in others. While Fischler believes that the liver may form urobilin he concedes that the intestines are the usual site of its synthesis. Meyer-Betz criticizes Fischler's conclusions and seeks to explain all of his results by assuming that some bilirubin reached the intestine by way of the blood because of the common occurrence of jaundice in bile fistula dogs. Wilbur and Addis have, in a measure, substantiated the work of Fischler. They observed an increased excretion of urobilin in the stool (and occasionally in the urine) of a dog that had cirrhosis of the liver. Further, they found that when the common bile duct was ligated the urobilin at first disappeared from the stool only to return later in diminished quantities, and that when a biliary fistula was produced in these animals the urobilin of the stool decreased but did not wholly disappear.

The arguments in favor of the so-called histogenic theory, which ascribes the formation of urobilin to the tissues, appear to be weak and inconclusive. The occurrence of urobilinuria after internal hemorrhage, for instance, is better explained by the enterogenous theory.

Determination.—The method of Wilbur and Addis is now commonly employed in this country for the determination of urobilin in the stool, bile and urine. The principal steps involved are as follows (the reader is referred to the original for all details): 10 c.c. of the 24-hour volume

of *urine* are added to 10 c.c. of saturated alcoholic zinc acetate solution and filtered. One c.c. of Ehrlich's solution is added to 10 c.c. of the filtrate. The reaction is allowed to progress in the dark for one-half hour. The solution is then diluted until the respective spectral absorption bands of urobilin and urobilinogen just disappear. The dilutions required give the value for 5 c.c. of urine. If this figure is multiplied by the factor, $\frac{\text{volume of urine c.c.}}{5}$ the number of dilutions for the 24 hours is obtained.

The *feces* are ground with water and made to a definite volume. An aliquot portion is extracted with 3 volumes of acid alcohol and then treated with zinc acetate and Ehrlich's reagent. The steps that follow and the computation are similar to those described for the urine. The average normal excretion in the stool per day is about 6,500 dilutions (Wilbur and Addis). Schneider (*a*) (1916) determines the urobilin in the *duodenal contents* by mixing 10 c.c. with 10 c.c. of the zinc acetate solution, and then filtering. (One drop of ammonia is added to the filtrate if it is not already alkaline.) One c.c. of Ehrlich's reagent is added to 10 c.c. of the filtrate. The dilutions are expressed in terms of 1,000 c.c. of bile.

Clinical Significance

An increased amount of urobilin in the urine is frequently observed in diffuse involvement of the liver as a result of fatty or parenchymatous degeneration, cirrhosis, new growth, abscess or even in the congestion due to heart disease. Wilbur and Addis record a daily excretion of from 1,100 to 3,000 dilutions of urobilin in the urine of patients suffering from cirrhosis, hemochromatosis or liver abscess. Owing to the variability of urobilin excretion in the urine it is desirable to continue the determinations over several days. Urobilinuria is quite common in the infectious diseases that produce degeneration of the liver as scarlet fever, lobar pneumonia, rheumatic fever, malaria, tuberculosis, etc. In biliary obstruction the amount of urobilin in the stool is proportional to the degree of patency of the common bile duct. Fischer and Meyer-Betz (*b*) (1912) studied the effect of administering fresh animal bile on the urobilin excretion in the urine. Under these conditions the urine of normal subjects contained little urobilin while patients suffering from liver disease excreted considerable amounts. Similar results were obtained when urobilinogen itself was administered. In the writer's limited experience the excretion of urobilin in liver disease has been quite irregular. At times no increase was observed; at times an increase occurred in the urine alone or in the feces alone while in some instances an increase in both urine and feces occurred (Bauman). It is conceivable that in hepatic conditions an increase in the urobilin of the stool may precede urobilinuria. The

increased excretion of urobilin in the stool of some cirrhosis patients was pointed out by Mueller (*a*) (1892).

A disease or condition causing an increased destruction of red cells is usually if not always accompanied by an increased elimination of urobilin in the bile, in the stool and sometimes in the urine as well. In secondary anemia the excretion of urobilin remains normal or subnormal while in pernicious anemia it may rise to 15 times the normal amount, hence urobilin estimations may serve to differentiate the two conditions.

Schneider (*a*) (1916) studied the urobilin in the duodenal contents of pernicious anemia patients. He found over 2,000 dilutions in pernicious anemia while in secondary anemia little or no increase could be detected. After splenectomy a decrease of the urobilin occurred. These results have been confirmed by Giffin, Sandford and Szlapka. Robertson (*b*) (1915) and McCrudden emphasize the diagnostic value of urobilin estimations of the stool, thus confirming the work of Wilbur and Addis. Most recently Howard and Hansmann, working in the writer's laboratory, studied the excretion of urobilin in the feces, urine and bile of a number of pernicious anemia patients. They conclude that the estimation of the stool is more reliable than that of the bile. Attempts to demarcate the 24-hour quantity of feces were unsuccessful. In pernicious anemia a marked increase of urobilin in the stool occurred even when the blood examination showed no abnormality. The urobilin was occasionally diminished during the remissions so frequently encountered in this disease.

Although obviously inaccurate the "quantitative" estimation of urobilin in the stool yields information which possesses considerable clinical value. On a priori grounds it would appear preferable to approximately determine the total daily excretion than that contained in a casual sample of bile; furthermore, it obviates the passage of the duodenal tube, a procedure which is sometimes disagreeable to the patient.

The diagnostic value of urobilin estimations is illustrated by the following case report:

An Italian, J. G. (history number 44,031), entered the Presbyterian Hospital in November, 1919, complaining of gastric distress and constipation which had lasted for 2 years but which was never accompanied by real pain, vomiting or diarrhea. During the 2 weeks prior to admission he had experienced a sudden attack of weakness and dizziness followed by the appearance of tarry stools and shortness of breath. During the period of illness he had lost approximately 25 pounds.

Physical examination showed evidences of neuroretinitis in both eyes occurring in an anemic man measuring about 5½ feet and weighing 143 pounds. The remainder of the examination was irrelevant. Radiographic examination and sigmoidoscopy were also negative.

The red cells numbered 2,000,000; hemoglobin was 40 per cent; white blood cells 6,800, of which 58 per cent were polymorphonuclear. The

blood smear showed irregularity in size and shape of the red cells, with central pallor and polychromatophilia on one occasion. The Wassermann test was negative. The gastric meal contained no free hydrochloric acid and a total acidity of 32. Lactic acid and occult blood were absent. The stool was repeatedly examined; occult blood was found on one occasion only. *The urobilin content of the stool was persistently subnormal; there was none in the urine.*

The patient was given two blood transfusions and was discharged after one month with the diagnosis of pernicious anemia. This diagnosis was made largely because of the negative radiographic examination.

During the following 6 months the patient's weight gradually increased by 15 pounds; and his blood recovered to the extent of about 5,000,000 red cells and 70 per cent of hemoglobin. He was readmitted in June, 1920, largely because of the uncertainty of the diagnosis and because his gastric symptoms had increased in severity. The red cells now numbered 5,200,000, and the hemoglobin 80 per cent. The 24-hour *stool contained 1,760 dilutions of urobilin; the urine contained 400 dilutions on one occasion and 1,088 on another.*

Fluoroscopy now showed a mass in the region of the cardiac end of the stomach, and this was confirmed by an exploratory laparotomy, which further revealed metastatic involvement of the liver and retroperitoneal lymph nodes.

In this case the severe anemia during the earlier period of the disease was probably caused by a profuse hemorrhage from the tumor. The low urobilin content of the stool militated against pernicious anemia and favored a new growth. The late occurrence of urobilinuria was due to the involvement of the liver.

Our ignorance of the fate of urobilin in the blood and tissues and its irregular excretion in the urine in cases of liver disease detract from its value as a functional test of liver efficiency. The interest aroused by the work of Wilbur and Addis in this country, and by that of Fischer abroad will stimulate investigation so that information relating to this phase of the urobilin problem will probably be furnished in the near future.

Resume

Urobilinogen and urobilin are almost exclusively derived from bilirubin by reduction by the bacteria of the large intestine. Urobilin is an oxidized and polymerized urobilinogen.

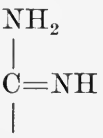
The determination of urobilin in the feces, urine and bile may be a valuable means of estimating the rate of blood destruction, thus aiding in the differential diagnosis of primary from secondary anemia; it may also serve to determine the functional state of the liver.

Creatin and Creatinin

LOUIS BAUMAN

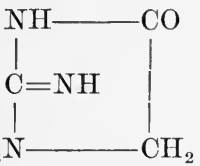
NEW YORK

Chemistry



Creatin, methylguanidoacetic acid ($CH_3N—CH_2COOH$), was first isolated from meat extract and named by Chevreul in 1835. Twelve years later Liebig isolated it from the muscle of various animals, analyzed it and converted it into its anhydride which he named creatinin. Creatin was synthesized from sarcosin and cyanamid by Volhard (1868), and from sarcosin and guanidin carbonate by Horbaczewski (*a*) (1885).

Creatin forms transparent prismatic crystals which contain one molecule of water. At room temperature it is soluble in water to the extent of 1.35 per cent. When heated with water or dilute mineral acids it is converted into creatinin. Conversely creatinin is converted into creatin when heated with calcium hydroxid solution.



Creatinin ( ) also occurs in the form of prismatic crystals which dissolve in water to the extent of 10 per cent; it is also more soluble in alcohol than creatin. Owing to its basic nature it is readily precipitated by the so-called alkaloidal reagents.

In watery solution creatin is slowly transformed into creatinin, the rate of transformation is slightly less than 0.5 per cent per day at 36° C. Under similar conditions creatinin is changed into creatin so that at the end of 11 months an equilibrium is established in either case. When these substances are dissolved in the urine a similar change takes place (Myers and Fine (*k*), 1915).

Both creatin and creatinin reduce alkaline copper solutions. When boiled with mercuric oxid they are oxidized to methylguanidin and oxalic

acid (Dessaignes). When creatin is oxidized with hydrogen peroxid in the presence of ferrous sulphate, glyoxylic acid is formed (Dakin (*c*)). Recently a new substance, methylguanidoglyoxylic acid, was obtained upon oxidizing creatin with mercuric acetate in watery solution (Bauman and Ingvaldsen). The successive steps in the oxidation of creatin may be formulated as follows:

1. $NH_2C(:NH)N(CH_3)CH_2COOH + O = NH_2C(:NH)N(CH_3)CHOHCOOH$.
2. $NH_2C(:NH)N(CH_3)CHOHCOOH + O = NH_2C(:NH)N(CH_3)COCOOH$.
3. $NH_2C(:NH)N(CH_3)COCOOH + H_2O = NH_2C(:NH)N(CH_3)H + COOHCOOH$.

The ease with which creatin is oxidized by metallic salts is noteworthy. The alleged occurrence of methylguanidin in the blood, muscle and urine may in reality be the result of oxidation of creatin by the mercuric or argentic salts which are ordinarily used for the purpose of isolation.

When picric acid is added to urine a characteristic potassium creatinin picrate is precipitated (Jaffé (*e*), 1886); this compound may be readily converted into the time-honored zinc chlorid salt according to the method of Benedict (*a*) (1914). In this manner relatively large quantities of creatinin (and creatin) may be prepared so that it has become readily accessible to most laboratories and is now used to prepare standard solutions for its quantitative colorimetric determination.

Jaffé (*e*) (1886) first noted that an alkaline solution of creatinin reduces picric acid to a reddish compound (probably picramic acid). Folin (*a*) (1904) proved that the intensity of the color was directly proportional to the amount of creatinin and therefore that this reaction was well adapted for its quantitative colorimetric determination. The publication of this method proved to be an incentive for numerous investigations of the physiological behavior of creatin and creatinin, since the former may readily be converted into the latter by relatively simple means.

The Creatin Content of Muscle and other Tissues

Creatin is a characteristic constituent of the muscle tissue of all vertebrates. In the skeletal muscle of the horse, for example, it forms approximately one-third of the total extractive nitrogen, the remainder being formed by carnosin and other compounds (Von Fuerth and Schwartz). Creatin is most abundant in voluntary muscle; there is less in heart muscle, and least in involuntary muscle. The following table gives the average percentage of creatin in the moist tissues of various animals:

Tissue	Animal	Creatin mg. %	Author
Voluntary muscle...	Rabbit	518	Myers and Fine (1913 (1))
	Dog	367	" " " "
	Cat	449	" " " "
	Kitten[1]	224	" " " "
	Human	393	" " " "
	Horse	380	Van Hoogenhuyze and Verploegh (1905)
	Pig	450	" " " " "
	Sheep	410	" " " " "
	Beef	440	" " " " "
	Rat	458	Myers and Fine (1915 (4))
	Fish[2]	500 to 700	Okuda
Liver	Dog	18	Beker
Heart muscle.....	Dog	216	"
Uterine "	Dog	30	"
	Beef	38	"
Testes	Beef	87	"
Brain	Beef	56	"
	Dog	56	"
Kidney	Beef	16	"
	Dog	14	"
	Pig	15	"
Brain	Dog	110-124	Janney and Blatherwick
Testes	Dog	181	" " "
Pancreas	Dog	18	" " "

[1] The creatin content of kitten muscle varies with the age of the animal.
[2] Various species of fish muscle were analyzed. The figures represent minimal and maximal values.

Denis (*e*) (1916) determined the creatin content of a relatively large number of samples of human muscle and found it to vary from 360 to 421 milligrams per cent. The muscle of children and that of persons dying of a wasting disease was usually found to be low in creatin.

As the creatin content of muscle is determined by the Folin method it was important to know if the color reaction was entirely due to this substance. By first transforming the creatin in muscle extract into creatinin and then quantitatively removing the latter by precipitation, Bauman and Ingvaldsen (*a*) (1916) were able to show that creatin alone was responsible for the Jaffé reaction.

The Origin of Creatin

A vast amount of experimental work has been done on this problem. The only other guanidin derivative which has been found in the animal body is the amino acid, arginin (alpha amino, delta guanido valerianic acid, $(NH_2C(:NH)NH—CH_2CH_2CH_2CHNH_2COOH)$. Arginin has been perfused and administered in various ways in order to see if it was converted into creatin. On the whole the results have not been uniform or conclusive. By analogy one might assume that arginin would first be oxidized to guanidoacetic acid or glycocyamin $(NH_2C(:NH)NH–CH_2$

COOH). This compound is converted into creatin when fed or injected into animals (Czernicki; Jaffé, 1906; Dorner; Bauman and Hines).

Van Hoogenhuyze and Verploegh (*a*) (1905) failed to observe an increase in creatinin excretion after the ingestion of proteins relatively rich in arginin. Myers and Fine (1905) report that the concentration of muscle creatin does not appear to be markedly influenced by the feeding of proteins having a high or low content of arginin. Jaffé (*f*) (1906) did not observe an increase in creatinin excretion after the injection of arginin into rabbits. Bauman and Marker also failed to note an increase of muscle creatin when arginin was circulated through dog muscle.

Thompson (*a*) (1917) administered arginin to ducks, dogs and rabbits and observed an increase in the elimination of creatin or creatinin and of the creatin content of the muscle. Inouye observed that arginin was converted into creatin when perfused through the liver of cats. In growing pigs the nature of the protein in the diet determines whether or not creatin appears in the urine (McCollum and Steenbock). Denis (*f*) (1917) has shown that the creatin excretion in hyperthyroidism may be much increased by the addition of protein to the diet. In children the creatin of the urine varies with the amount of protein in the diet (Denis and Kramer). Creatinuria in women follows the ingestion of large amounts of protein (Denis and Minot (*a*)).

Riesser observed an increase in muscle creatin and in the creatinin excretion of rabbits after the injection of cholin and betain.

Harding and Young found that arginin was without effect on the creatin excretion of growing dogs but that a variation in the cystin content of the diet was followed by a similar variation in the creatin elimination.

Most recently Wishart observed an increase in muscle creatin following the injection of guanidin salts into cats, dogs and frogs. The assumption is that guanidin is detoxicated by conversion into creatin.

In the foregoing experiments the factor of creatin destruction by the tissues must not be overlooked. Creatin may be synthesized from a precursor but subsequently destroyed.

Creatin Metabolism

Muscle.—Before discussing this subject it may be well to remind the reader that the experimental results obtained by different investigators are often conflicting and therefore hard to reconcile with one another.

Considerable evidence seems to show that creatin is a product of muscle metabolism. Its preponderance in muscle suggests that it results from metabolic processes peculiar to this tissue (Pekelharing). Muscle creatin increases with an increase in muscle tonus and conversely paralyzed muscle

is low in creatin (Pekelharing and Van Hoogenhuyze (*a*), 1909; Jansen (*b*)). Voluntary muscle has an affinity for creatin, for when it is injected into rabbits the creatin content of their muscles is increased by 5 per cent (Myers and Fine (*e*), 1913).

The constancy of the creatin content of muscle of a given species of animal under uniform conditions of diet was first pointed out by Myers and Fine (*c*) (1913). During starvation or carbohydrate abstinence the creatin content of muscle at first increases and then progressively decreases with the length of the fast (Mendel and Rose (*b*), 1911). The muscle of rabbits that had fasted for 6 days contained 0.55 per cent of creatin, while that obtained from rabbits that had been starved for 24 days contained 0.36 per cent (Myers and Fine (*d*) 1913). The decrease in creatin is explained by the loss of this substance through the urine.

Benedict and Osterberg maintained phlorhizinized dogs in approximate nitrogen equilibrium by feeding creatin free protein. Under these conditions the excretion of creatin continued unchanged, and in spite of the relatively large quantity lost in the urine the muscle of these animals actually contained more than that of normal dogs. The authors conclude that the creatin excreted in the urine is not dependent on the amount of body tissue destroyed, that it is not derived from muscle creatin, and further that creatin is probably formed in large amounts and is normally utilized or destroyed for the greater part. The creatinin of the urine can only account for a small part of the creatin that is normally katabolized. Folin and Denis (*h*) (1914) found that when creatin was injected into cats it was absorbed by the muscles to an extraordinary degree. They believe that living muscle does not contain free creatin and that that found on analysis is a post-mortem product. The vital combination must be a very loose one to be sure.

According to several authors creatin is not destroyed during aseptic or antiseptic autolysis of muscle (Denis (*e*), 1916; Mellanby (*a*)). Myers and Fine (*k*) (1915) find that no destruction of creatin or creatinin occurs when rabbit muscle is permitted to autolyze (under aseptic conditions) at body temperature. On the other hand the work of Hoagland and McBryde seems to show that during aseptic autolysis of beef muscle creatin at first increases and then decreases.

Blood.—Normal blood contains between 3.5 and 6 milligrams of creatin per 100 c.c. (Folin and Wu). In nephritis as much as 31.7 mgs. have been observed (Myers and Fine(*g*), 1915). Though the concentration of creatin in the blood is higher than that of creatinin the former is usually not excreted by the kidney while the latter is a normal constituent of the urine. In other words the renal threshold for creatinin is lower than for creatin. The concentration of creatin in the plasma is lower than in whole blood (Hunter and Campbell (*b*)).

Urine.—Under normal conditions creatin is absent from the urine of men when living on a creatin free diet; it is constantly present in the urine of children and frequently occurs in the urine of women. Powis and Raper have shown that children eliminate more creatin during the day than at night. In the young the supply of carbohydrate and fat appears to be unable to meet the demands of growth and maintenance, and as a consequence muscle tissue disintegrates, creatin is liberated and appears in the urine. The frequent occurrence of acetonuria in children and the rapidity with which the glucose content of their blood is lowered during starvation are further indications of a limited supply of glycogen (Sawyer, Stevens and Bauman). The occurrence of creatin in the urine of children may also be due to a diminished ability to destroy it (Krause (*b*), 1913; Gamble and Goldschmidt (*a*), 1919). In infants the increased excretion of creatin when they are on a pure milk diet may be due to the creatin present in the milk and not to the protein therein (Gamble and Goldschmidt (*b*), 1919).

Sawyer, Stevens and Bauman observed that the increased excretion of creatin which occurs in children when deprived of carbohydrates is usually followed by a period of creatin retention upon resumption of the normal diet. It appears as if the body retained creatin with great regularity under these circumstances.

The alleged occurrence of creatinuria after menstruation (Krause (*a*), 1911) has not been confirmed by M. S. Rose, who found no definite relation between the creatin output and the sexual cycle, nor was creatin excretion affected by protein feeding. In normal pregnancy the excretion of creatin is usually less than 20 per cent of the creatinin excretion (Van Hoogenhuyze). A pregnant woman excretes about 170 mgs. of creatin and the same woman during the lying-in period eliminates about 470 mgs. (Van Hoogenhuyze and ten Doesehate). After cesarean section an increased elimination of creatin occurs even when the uterus has been removed at the time of operation (Mellanby (*b*), 1913; Morse). F. G. Benedict (*c*), and F. G. Benedict and Diefendorf first noted the occurrence of creatin in the urine of starving men and women. Mendel and Rose (*a*) (1911) found creatin in the urine of adult animals when they were deprived of carbohydrates and began to break down their body proteins. Certain animals having small reserves of glycogen and fat, as the rabbit, will excrete creatin after a short fast, while others with large stores of fat, as the pig, can be fasted for from 14 to 16 days without excreting creatin (McCollum and Steenbock). In this respect the human being and dog occupy intermediate positions. Mendel and Rose (*a*) (1911) found that rabbits began to excrete creatin on the second day of starvation and that the amount excreted gradually rose until death. Depriving the tissues of carbohydrates by means of phlorhizin poisoning also leads to creatinuria (Mendel and Rose (*a*), 1911; Cathcart and Taylor).

From the foregoing one might conclude that creatinuria regularly accompanies undernutrition, whatever the cause. This is actually the case. Diabetes, carcinomatosis, hyperthyroidism, fevers, incessant vomiting and other wasting conditions are usually accompanied by the appearance of creatin in the urine. Feeding thyroid substance increases the metabolic rate and leads to the elimination of creatin (Krause and Cramer). Shaffer (*a*) (1908) found that of 10 cases of hyperthyroidism 8 exhibited creatin in the urine. Denis (*f*) (1917) has shown that the creatin excretion in this condition is increased by feeding a high protein diet. As hydroxbutyric and acetoacetic acids often accompany creatin in the urine it has been supposed that a causal relationship exists between acidosis and creatin excretion. Underhill (*k*) (1916) noted that rabbits began to excrete creatin when they were fed on acid producing diets or when hydrochloric acid itself was administered. In both series of experiments the supply of carbohydrates was sufficient and the protein per se was without influence. Underhill (*l*) (1916) also found that the administration of alkalies diminished the creatin output during the early days of starvation. In phlorhizin glycosuria, however, alkali administration was without effect (Underhill and Baumann). McCollum and Hoagland (*a*) (1913) observed that pigs eliminated creatin when fed on fats, water and neutral salts, but failed to do so when the salts were alkaline. Considering all the known facts pertaining to this phase of the subject it appears unwise at present to assume a causal relationship between acidosis and creatinuria.

Creatinin Metabolism

Muscle.—Skeletal muscle contains from 5 to 15 mgs. of creatinin per 100 grams of moist tissue (Myers and Fine (*i*), 1915; Folin and Denis (*g*), 1914), that is, from 5 to 10 times the amount which is present in the blood which circulates through it. Shaffer (*b*) (1914) holds that this is an argument in favor of the view that creatinin is formed in muscle tissue. The rate of conversion of creatin into creatinin in autolyzing muscle is proportional to the temperature and is 3 times more rapid than in watery solution.

Blood.—The blood of normal individuals contains from 1 to 2 mgs. of creatinin per 100 c.c. (Folin and Denis (*g*), 1914). In nephritis relatively large quantities, as much as 33 mgs. have been reported. In pathologic conditions of the kidney uric acid and urea are retained before creatinin and elevations of the last above 5 mgs. indicate a grave prognosis except in acute renal inflammations (Myers and Lough).

Urine.—In a classical article published in 1905, Folin showed that the excretion of creatinin on a meat free diet was constant for each individual

and independent of the exogenous metabolism and the total nitrogen excretion. Shaffer (*a*) (1908) confirmed these observations and found that the hourly excretion of creatinin was also uniform. This constancy of creatinin elimination has been used to control the accuracy of the 24-hour urine collection. The daily creatinin excretion for an adult man lies between 1 and 2 grams. From the viewpoint of quantity it is second in importance to urea. A normal man excretes between 7 and 11 mgs. of creatinin nitrogen per kilo of body weight; this has been named the creatinin coefficient by Shaffer (*a*) (1908). It is apparently a function of the mass of active muscle tissue for stout and elderly people, and women often have values below 7. The coefficient of the dog averages 8.4. Myers and Fine (*c*) (1913) have studied the relation of the creatinin coefficient to the total creatin content of the body. In the case of the rabbit this is quite constant, averaging 44.7 mgs. of body creatin to 1 of creatinin in the urine. The daily output of creatinin represents a conversion of about 2 per cent of the total creatin present in the body. The creatin content of the rabbit per kilogram is about one-third higher than that of man, and its creatinin coefficient is proportionately higher, that is, 14.

The creatinin excretion of women is lower than that of men. Tracy and Clark found the average creatinin coefficient of 26 women to be 5.8. According to these authors the low coefficient of women is due to their relatively inferior muscular development. Hull found the average creatinin excretion to range between 670 and 880 mgs. Muscular activity has no effect on creatinin excretion (Van Hoogenhuyze and Verploegh (*b*), 1908; Shaffer (*a*), 1908).

During starvation there is a gradual decrease in creatinin in the urine along with an increase in creatin (Catheart (*a*), 1907; Howe, Mattill and Hawk (*b*); Hunter, 1914). Pigs that were fed on a liberal amount of carbohydrate, salts and water reached a stage when the creatinin accounted for 18 per cent of the total nitrogen in the urine (McCollum and Hoagland (*a*), 1913). Fevers cause an increase in urinary creatinin (Van Hoogenhuyze and Verploegh (*b*), 1908; Klercker (*c*), 1909; Leathes (*a*)). Myers and Volovic observed that the increase was proportional to the height of the temperature.

Creatin is often present in the urine in conditions associated with dissolution of muscle tissue, and then the creatinin is usually found to be decreased (Levene and Kristeller). Spriggs reported a very low creatinin excretion in 2 cases of muscular dystrophy and also in a case of amyotonia congenita. In progressive muscular dystrophy, McCrudden and Sargent observed large quantities of creatin in the urine with a constant creatinin elimination.

In wasting or atrophy of muscle the creatin eliminated in the urine is probably derived from the disintegrated muscle fibres.

The Fate of Administered Creatin or Creatinin

A number of investigators have attacked this problem. The experiments of Myers and Fine (*e*) (1913) are fairly representative. These observers found that when creatin was injected into rabbits a small portion was deposited in the muscles, and from 25 to 80 per cent, depending on the amount injected, could be recovered from the urine. When creatinin was administered an average amount representing 80 per cent of that injected was found in the urine and the remainder was deposited in the muscles. When creatin was fed to man a slight increase in creatinin elimination occurred which accounted for from 3 to 4 per cent of the ingested substance; from 0 to 39 per cent, again depending upon the amount administered, appeared in the urine unchanged (Myers and Fine (*h*), 1915). Many of the other investigators obtained similar results. See Folin (*e*) (1906), Klercker (*a*) (*b*) (1906, 1907), Wolf and Shaffer, Van Hoogenhuyze and Verploegh (*b*) (1908), Pekelharing and Van Hoogenhuyze (*b*) (1910), Foster and Fisher, Towles and Voegtlin, Folin and Denis (*a*) (1912) and Krause.

Summarizing, it may be said that when creatinin is administered it is excreted almost quantitatively, whereas creatin is only partly excreted, the major portion being probably destroyed in the body. Only a small percentage of the administered creatin is excreted as creatinin. There is no evidence that creatin is converted into urea. On a high protein diet a smaller amount of administered creatin is retained than on a low diet. According to Krause (*b*) (1913) children are less able to destroy creatin than adults.

Gibson and Martin observed that creatin was promptly excreted when administered to patients suffering from progressive muscular atrophy.

Résumé

The creatin content of muscle is fairly constant for a given species of animal under uniform conditions of diet.

Muscle creatin is diminished during carbohydrate privation. This change is ascribed to the loss of creatin in the urine.

The normal excretion of creatin by children and young animals in general is probably due to their relatively high planes of metabolism and their small reserves of glycogen. In the absence of carbohydrate, fat, and protein to a lesser extent are called upon to supply the body requirements; under these circumstances muscle tissue is disintegrated, creatin is liberated and excreted in the urine.

The precursor of creatin has not been definitely established. Creatinin

is probably derived from creatin, that is, a definite percentage of the body creatin is daily converted into creatinin. The creatinin excretion is proportional to the bulk of active muscle tissue. The daily amount of creatinin excreted by a given individual is constant under widely varying conditions. It is increased during fever and diminished during starvation and during periods of muscle disintegration.

Creatinin is eliminated by the kidneys with great facility and is only retained in the blood in advanced disease of the kidneys. When creatinin is fed or injected it is almost quantitatively eliminated, whereas creatin under similar circumstances is largely destroyed in the body.

Normal Fat Metabolism

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Introductory

In the course of the great development which has taken place in biochemistry during the last few years our knowledge of metabolism has been greatly extended, especially in the fields of the proteins and the carbohydrates. Comparatively little has been added to that of the fats, for which the main reason is the difficulty of chemical examination and determination. The fats are relatively inert substances which do not lend themselves readily to reactions which may be used as a basis for their study, and as a result there is not the same background of exact chemical knowledge as in the case of the proteins and carbohydrates. Another reason is that in their function as stored material, the part which they and their derivatives play in the life processes of the cells has been obscured, and all the more so since the comparative inertness of the fats would seem to render them unfit to take part in the delicately balanced reactions of living protoplasm. Just the opposite may, however, be said of certain of their derivatives such as the phospholipoids, members of which group are among the most reactive substances found in living beings. In fact, so great is their tendency to break up, to oxidize, to combine with a great variety of substances that it is with extreme difficulty that they can be prepared pure enough for analysis. In recent years methods have been devised for the study not only of the fats but of the more important related substances in living organisms, and the result has been an aroused interest in the whole field. With the accumulation of data has come the realization that the study of the metabolism of the fats, meaning essentially that of the fatty acids, involves many if not all of the compounds of the fatty acids, and that only by a consideration of the whole group of compounds can a true picture of the metabolism of fat be obtained. For this reason it has appeared necessary to reclassify the fats and related substances on the basis of their relationship to the fatty acids in metabolism, and a brief outline of such a classification with a short description of some of the more important members is given below. For a more detailed

discussion of the classification and of the members the reader is referred to other sources (Bloor (*i*), 1920; Leathes (*c*), 1910).

The Lipoids

Naturally occurring compounds of the fatty acids, together with certain substances found naturally in chemical association with them.

The group is characterized in general by insolubility in water and solubility in "fat solvents," chloroform, benzol, etc.

Simple Lipoids.—Esters of the fatty acids with various alcohols.

Fats.—Esters of the fatty acids with glycerol. (Fats which are liquid at ordinary temperatures are called oils.)

Waxes.—Esters of the fatty acids with alcohols other than glycerol. Beeswax, lanolin, cholesterol oleate.

Compound Lipoids.—Compounds of the fatty acids with alcohols but containing other groups in addition to the alcohol.

Phospholipoids.—Substituted fats containing phosphoric acid and nitrogen. Lecithin, cephalin, etc.

Glycolipoids.—Compounds of the fatty acids with a carbohydrate and nitrogen but containing no phosphoric acid. Cerebron.

(*Amino lipoids, Sulpho lipoids, etc.*—Various groups which may be added as soon as they are sufficiently well characterized.)

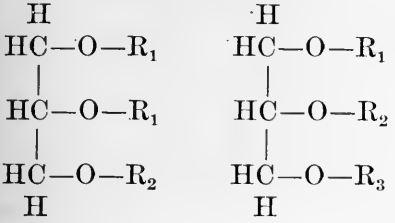
Derived Lipoids.—Substances, derived from the above groups by splitting, which have the general properties of the lipoids.

Fatty acids of various series.

Sterols.—Alcohols, mostly large molecular solids, found naturally in combination with the fatty acids and which are soluble in "fat solvents." Cetyl alcohol ($C_{16}H_{33}OH$), myricyl alcohol ($C_{30}H_{61}OH$), cholesterol ($C_{27}H_{43}OH$).

Simple Lipoids.—*The Fats.*—Esters of the triatomic alcohol glycerol. They are commonly called fats when they are solid at ordinary temperatures and oils when liquid. Of the lipoids these are the most widely distributed in nature, the most important from the point of view of nutrition and the best understood chemically. As ordinarily occurring, they are triatomic esters, i. e., all three of the hydroxyl groups of the alcohol are replaced by fatty acids. Diatomic and monatomic esters are occasionally found but usually only where metabolic processes are in active progress as in germinating seeds and during fat digestion. The fatty acids in combination with a single glycerin molecule may be either all the same—producing simple glycerides—or may be different, producing mixed glycerides. As the knowledge of the chemistry of the fats increases it becomes evident that mixed glycerides are of much more frequent occurrence than was previously supposed—a fact which is of considerable

interest from a biochemical point of view because of the potential optical activity of many of these mixed esters, since optical activity is recognized as a property closely connected with life processes. Thus



(R_1 R_2 R_3 being different fatty acid radicals)

should from the structure be optically active. Up to the present time no optically active fats have been found in nature or been prepared synthetically, which may mean merely that present day methods of preparation and separation of isomers are not adequate for the purpose. On the other hand many of the phospholipoids are optically active and contain different fatty acids in combination, and since there is good reason to believe that the phospholipoids are stages in the metabolism of the fats and are known to be constituents of living tissues, the inference is that while the fats themselves may not take part in life processes they are readily changed into substances which do.

Waxes.—Distinguished from the fats by the fact that the alcohol in combination is not glycerol. These are substances widely distributed in nature but in amounts much smaller than the fats. They are characterized in general by great chemical inertness; they are much more difficult to oxidize or to hydrolyze either by enzymes or other agents. The constituents of the waxes have been completely worked out in but few cases, so that our knowledge of the chemistry of these substances is very fragmentary. The alcohols found in combination in the waxes are mostly of large molecule (see under Sterols), and the fatty acids are also generally large molecular and either saturated or containing hydroxyl groups. Common examples of the waxes are:

Beeswax.—A mixture of many substances of which the best-known ones are esters of myricyl ($C_{30}H_{61}OH$) and ceryl ($C_{26}H_{53}OH$) alcohols with palmitic ($C_{16}H_{32}O_2$), cerotic ($C_{26}H_{52}O_2$) and melissic ($C_{30}H_{60}O_2$) acids and much free cerotic acid.

Cetin.—The ester of cetyl alcohol ($C_{16}H_{33}OH$) and palmitic acid.

Wool Wax (Lanolin).—Contains esters of cholesterol derivatives with various fatty acids.

Cholesterol esters of palmitic and oleic acids are present in blood.

Compound Lipoids.—*Phospholipoids.*—Compounds of the fatty acids and glycerol containing phosphoric acid and nitrogen. They are widely distributed in nature, being constant constituents of living cells. They

may be regarded as phosphorized fats—glycerides in which one fatty acid has been replaced by a substituted phosphoric acid. On hydrolysis they yield fatty acids, glycerophosphoric acid and a basic substance, which in the case of lecithin is mainly cholin and in cephalin probably aminoethyl alcohol.

In Cuorin, a phospholipoid from heart muscle, the proportion of phosphoric acid to fatty acid is greater than in lecithin.

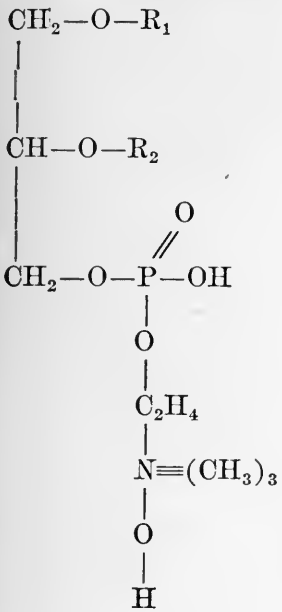
Since satisfactory chemical characterization and identification of most members of this group has not yet been made reference will be made to only a very few.

In general they are very active chemically, undergoing rapid changes in air and light, becoming colored and rancid. They are not soluble in water in the ordinary sense, but mix with it, forming opalescent colloidal suspensions. They are readily hydrolized by many reagents as well as by the lipases and esterases and even by boiling with alcohol (Erlandsen). They form combinations readily with many substances, as, for example, with proteins and carbohydrates, but these combinations are unstable and of inconstant composition, so that it is doubtful whether they are true chemical compounds. The similarity in chemical composition indicates a close relationship to the fats; the constant occurrence in quantity in living active cells, the ready reactivity to oxidation, hydrolysis and combination with other tissue constituents and, above all, the miscibility with the universal solvent, water, indicate that the phospholipoids are the intermediate step through which the fats pass before being finally utilized. The fatty acids obtained from the phospholipoids were thought by the earlier investigators (Hoppe Seyler, etc.) to be the same as those in ordinary animal fats, i. e., stearic, palmitic and oleic, but recent work, particularly that of Leathes (*c*), 1910, Hartley (*a*), 1907-08, Erlandsen and MacLean have shown that the earlier supposition is not correct and that, if care be taken to avoid oxidation, mainly unsaturated fatty acids are obtained.

The Lecithins.—The best known of the phospholipoids. They are characterized by their insolubility in acetone—a property which is made use of in their separation. They are readily soluble in other fat solvents and form a colloidal solution with water. Most members of this group are very sensitive to chemical change, so that it is almost impossible to prepare them in pure form. In addition to their chemical sensitiveness they possess, in a higher degree than most other organic compounds, the power of uniting with other substances such as salts (NaCl), compounds of the heavy metals as Pt and Cd, and with many organic substances such as alkaloids, toxins (snake venoms), carbohydrates and proteins. These combinations are not of constant composition and are broken up by relatively gentle treatment, e. g., boiling with neutral solvents, and it is therefore a question whether they are true chemical compounds or merely physical (adsorption) mixtures. This power of combination is of great

significance in the consideration of these lipoids as constituents of living matter.

The chemical formula of a typical lecithin which embodies our knowledge of its composition at the present time is:



As indicated by the formula the fatty acid groups (R_1 and R_2) are generally different and the compounds are optically active. The fatty acids are often unsaturated, particularly in the lecithins from the active organs as heart, liver, etc.

Cephalins.—These differ from lecithins in being difficultly soluble in alcohol and in containing a different basic group, the exact nature of which is unknown, but which is believed to be amino-ethyl alcohol. They are widely distributed in the body and, according to Thudicum, are the main phospholipoids of the brain. They have recently received a good deal of attention because of their connection with blood coagulation (Howell). MacLean has shown that they are formed rather easily from lecithin and that one of the difficulties in preparing pure lecithin is its tendency to lose its methyl groups and pass over into cephalin.

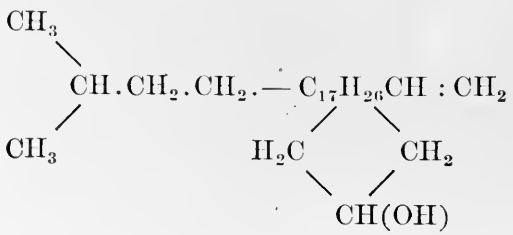
Glycolipoids.—These substances, characterized by their content of carbohydrate, are less understood than the phospholipoids. The only one which has been well studied is the cerebrone of Thierfelder, a constituent of brain tissue. The carbohydrate is galactose, the fatty acid a higher isomer of stearic acid, and there is also a basic substance known as sphingosine.

Derived Lipoids.—*Fatty Acids.*—The fatty acids found combined in the fats include practically all the known fatty acids of the various series which contain even numbers of carbon atoms arranged in straight chains. Fatty acids of odd numbers of carbon atoms are so rare that their natural origin is questionable, while branched chains are unknown. A few acids

of the benzene series should perhaps be included since they are found in certain natural oils (chalmougra oil, etc.). The fatty acids most frequently found in animals are palmitic, oleic and stearic acids. In active tissues fatty acids of the linoleic and possibly of still more unsaturated series are to be found, while in the brain hydroxy acids are present, together with a great variety of unsaturated fatty acids.

In milk are to be found all known even numbered members of the acetic acid series, beginning with butyric and ending with arachidic.

Sterols.—This group includes the alcohols found naturally in combination with the fatty acids in the waxes. They are generally inert substances of large molecule, mainly of the straight chain monatomic group of alcohols. The notable exceptions to this rule are cholesterol and related substances,—secondary alcohols belonging to the terpene series; most sterols occur in the free as well as in the combined form. The more important members of this group are cetyl ($C_{16}H_{34}O$) and octodecyl ($C_{18}H_{38}O$) alcohols in spermaceti, ceryl alcohol ($C_{26}H_{54}O$) in Chinese wax, myricyl alcohol ($C_{30}H_{62}O$) in beeswax, cocceryl ($C_{30}H_{62}O_2$) in cochineal wax and the cholesterol group containing cholesterol ($C_{27}H_{44}O$) in most animal tissues and fluids, the isomeric phytosterol similarly distributed in plants, isocholesterol ($C_{26}H_{46}O$) and a number of others more or less well characterized. Of these, the only one which calls for extended discussion is cholesterol. According to our present information it is a monatomic secondary alcohol with a terminal vinyl group. The nucleus probably contains four to six carbon rings and belongs in the general group of terpenes. The details of structure are illustrated in the formula:



In the free form or as esters with the fatty acids it is widely distributed in animal tissues and fluids and either as such or as various derivatives (the bile acids have been so regarded) it is probably of great importance in animal metabolism.

Of the fatty acids those most frequently found in combination with cholesterol are oleic and palmitic acids.

Cholesterol is a colorless, odorless substance crystallizing in thin plates, insoluble in water, soluble in fats and fat solvents, melting at 148.5° C., and is optically active. Specific rotation $[\alpha]_D^{51} = -29.92$. The corresponding alcohol in plants is phytosterol which, according to Gardner, changes to cholesterol during intestinal absorption in animals.

Closely related substances found in animals and probably derived from cholesterol are coprosterol in feces and isocholesterol in skin and hair waxes.

Fat Digestion and Absorption

The Stomach.—*Digestion.*—Fat splitting enzymes (lipases) may appear in the stomach from either of two sources—as part of the gastric secretion or by regurgitation from the intestine. The presence in the stomach of secretions from the small intestine, especially bile, has been known clinically for many years, and while the tendency has been to minimize the influence of these secretions on fat digestion it is realized that under suitable conditions splitting of fats in the stomach may assume considerable proportions. Cannon has shown that fats slow the emptying of the stomach by inhibiting the production of acid, also that the pylorus is kept closed by the presence of acid on the intestinal side of the sphincter. In the absence of acidity the pylorus may relax or open and allow regurgitation of intestinal contents including lipases by reverse peristalsis, and under the conditions of low gastric acidity considerable lipolysis would take place. Boldyreff found that after a meal rich in fat there is a reflux of pancreatic secretion into the stomach.

Quite aside from the regurgitated intestinal material the stomach has a lipase of its own, a fact which was claimed many years ago by Ogata and other observers. Their work received little attention until it was confirmed by Volhard and his pupils. Volhard's work stimulated investigation and discussion and the existence of a gastric lipase has been a much debated topic since that time. One difficulty has been to rule out the possibility of intestinal lipase, and when this has been successfully accomplished the low values obtained for lipolysis by pure gastric juice have thrown doubt on its existence in amounts worthy of consideration. Volhard found undoubted digestion of the emulsified fat of milk and egg-yolk both by gastric juice obtained by siphon and by glycerin extract of the mucous membrane of the fundus, and his findings have been confirmed by several workers since (Davidsohn, 1912), while London and others were unable to demonstrate lipase in gastric juice from a Pawlow stomach. Davidsohn has compared the properties of gastric and of pancreatic lipase and found differences in their optimum reaction. For pancreatic lipase the optimum reaction was $\overset{+}{H} = 1 \times 10^{-8}$, while for stomach lipase it was 2×10^{-6}—also that pancreatic lipase was much more sensitive to sodium fluoride.

The probable reason for the conflicting results regarding gastric lipase has recently been found by Hull and Keeton, who studied the lipase in gastric juice obtained from Pawlow stomachs and in normal stomachs, of which the pylorus had been ligated and the flow of secretion stimulated by gastrin and by food. They found that the gastric lipase was sensitive

to acid, being destroyed by a fifteen minutes' exposure to 0.2 per cent hydrochloric acid, and that if the acidity was reduced either by ordinary neutralization with alkali or by protein a fairly good lipase action could be demonstrated (about five times as great as that of the succus entericus). The practical bearing of their work was to indicate that after a meal and before the stomach acidity had reached a value high enough to destroy the lipase (being kept down by the proteins of the food) considerable fat splitting might take place, at least of emulsified fats.

The sum of the work to date leaves little doubt that a lipase is secreted by the stomach. Whether there is much fat splitting will depend on a number of factors among which are the following: (a) The acidity of the stomach contents—high acidity destroying and lower acidity down to a certain point inhibiting the activity of the gastric lipase. The degree of acidity is dependent on the amount of acid secreted and on the amount of neutralizing substance (mainly protein) present. The presence of fat inhibits acid secretion. (b) The state of division of the fat. Since the lipase and the fat have no mutual solvent, the splitting can take place only at the surface of the fat particles, and unless these are very small and the surface correspondingly great (as in an emulsion) not much splitting is likely. The acidity of the stomach is probably rarely weak enough to permit the formation of soap emulsions so that the lipolytic activity of the gastric juice would be confined to natural emulsions as milk, etc. The splitting of the fat in these emulsions may be very considerable (Volhard). (c) The length of time the fat remains in the stomach. The presence of much fat slows the passage of food from the stomach (Cannon), giving more time for the gastric lipase (and also the regurgitated pancreatic lipase) to act.

Absorption.—Klemperer and Scheuerlen, by ligating the intestine of dogs below the pylorus and weighing fat before and after 3 to 6 hours in the stomach, found that none had been absorbed. The objection might be raised in this case, as in many similar ones, that the operative procedures were responsible for the failure. Histological observations from von Kölliker onwards have demonstrated fat droplets in the gastric epithelium although none were seen in the lymphatics. Weiss believed that absorption into the epithelia was confined to young animals, in which belief he is opposed by Greene and Skaer, who found absorption (into the epithelium) in both young and old animals and also that the amount of absorbed material (observed by staining) and the depth of penetration depended on the length of stay of the fat in the stomach. The histological picture was found by these observers to resemble strongly the appearance of the intestinal mucosa during fat absorption. After the fat left the stomach the cycle reversed and the fat disappeared (back into the stomach?).

Mendel and Baumann studied the absorption of fat by the stomach

histologically and chemically, and confirmed in general the work of Greene and Skaer, although in some animals they found no penetration. They found no change in the fat content of the blood as a result of the presence of fat in the stomach, but they point out that the absorption would be necessarily slow and that the fat may have been removed from the blood as fast as absorbed. That absorption of other substances went on normally in these same animals was shown by tests with iodids. On feeding fat stained with Sudan III no color could be observed in the lymph or in the blood.

The Intestines.—*Passage from the Stomach.*—When the amount of fat in the food is small it probably does not affect appreciably the rate of emptying of the stomach, which proceeds normally as described by Cannon—the pylorus opening under the stimulus of a sufficient acidity of the food on the gastric side and closing when the acid food reaches the intestinal side of the opening valve. When the amount of fat in the food is large the gastric secretion is inhibited, the amount of acid produced is lessened, and it therefore takes longer for the food to reach the degree of acidity necessary to bring about the opening of the pylorus. The rate of emptying of the stomach is thus slowed and the rate at which the fat reaches the intestine is lowered. When, however, the fat is taken in liquid form (as oil) or suspended in a liquid, as in milk, it may pass immediately through the stomach like other liquids.

Thus in all cases except where the fat is taken in quantity in the form of oil (an unusual condition) it is passed into the intestine in small portions. When it reaches the intestine in large quantities diarrhea may be produced either through action of the fat itself or more probably as the result of irritation produced by the abnormally large amounts of soaps formed. One result of the normal functioning of the gastric mechanism is therefore the delivery of the fats to the intestine in small amounts, which has a direct bearing on the question as to the form in which it is absorbed from the intestine, since under these circumstances the chances are that the fat will be completely hydrolyzed in the presence of the relatively large amounts of pancreatic and intestinal lipases which it encounters. When the amount of fat in the food is so large that there is great inhibition of gastric secretion the pylorus appears to lose its tone after some hours and allows the passage of intestinal contents—bile and pancreatic secretion with its lipase—to pass into the stomach, where considerable hydrolysis of the fats may take place. Boldîreff has shown that this regurgitation may be made to take place readily in humans by feeding fat containing fatty acid.

Natural food fat always contains some free fatty acid and the amount is increased during the processes of cooking and by whatever lipolytic action occurs in the stomach, so that by the time the fat reaches the intestine there is probably always a considerable quantity of free fatty

acid present which, uniting with the alkali of the intestinal secretions, produces soap enough to emulsify the whole amount and thus prepare it for the action of the intestinal lipases.

The Lipases of the Intestinal Tract and Digestion.—Lipases are secreted into the intestine mainly by the pancreas, although Boldîreff has found that the intestinal secretions contain a lipase acting on emulsified fat which is different from pancreatic lipase in that its action is not accelerated by bile. Boldîreff tested lipolytic action with monobutyrin, milk and olive oil (Jansen objects to the use of monobutyrin because it is split by water alone and because in all probability a different ferment, monobutyrinase [an esterase] is involved). The lipolytic activity of intestinal juice is ordinarily slight, and in the presence of normal pancreatic secretion is probably not an important factor in fat digestion. Bile increases its activity. The flow of secretion in fasting is small and is increased by the presence of food, secretin, acids and soaps. In general, the amount of secretion is less the farther away from the duodenum it is collected.

The excitants for the secretion of pancreatic juice are normally acids (H), fats and water; alkalies have a retarding action. Acids act probably by the formation of secretin, rather than by reflex action on the intestine, as Pawlow believed, although stimulation of the nerve supply will cause secretion. Fats are found to act as excitants only when partially saponified, and soap is probably therefore the active substance—which is rendered the more likely since soap has been found by Fleig to produce a secretion. By the time it reaches the intestine food fat normally contains enough free fatty acid to form a considerable amount of soap with the alkali of the intestinal secretions. Water acts mainly indirectly by stimulating acid gastric secretion. The nervous system undoubtedly also plays an important part in pancreatic secretion not only as a regulator but also in the production of the secretion (Bylina, 1911).

The amount of pancreatic juice secreted in a 24-hour period has been found to vary greatly, the average from normal dogs (Pawlow and coworkers) obtained by pancreatic fistula being about 22 c.c. per kilo per 24 hours. For human beings the amount is reported to be about 600 c.c. per day.

The pancreatic lipase (steapsin) hydrolyzes the fats to fatty acids and glycerol, an action which is reversible, as was first reported by Pottevin, later confirmed by Taylor and Hamsik (*a*) (1909), and finally more conclusively by Foâ (*a*), who determined the exact conditions by which an excellent synthesis may be accomplished. By using oleic acid homogenized with glycerol and mixed with glycerol extract of pancreas (therefore with excess of glycerol) he was able to get a synthesis of about 62 per cent of the oleic acid used in 50 hours at 38° C. The compound formed was mainly the triglyceride. Armstrong and Gosney have made an exact study

of the reaction, using castor bean lipase. They found that, proceeding in either direction with the glycerid or with glycerol and oleic acid in the proportions found in the natural glycerid the equilibrium point was reached when about 40 per cent of the acid was combined. During the synthesis the compounds formed were apparently mainly diglycerids. During the hydrolysis with excess of water and near the beginning a small amount of a lower glycerid was present, but as the action continued the molecule was completely hydrolyzed. When only a small proportion of water was present a greater proportion of mono- and diglycerids was produced. Conversely when the synthesis is effected in the presence of water more of the triglycerid is formed. Synthesis in the presence of extra glycerol results as would be expected in a proportionately greater combination of fatty acids with the formation of more of the lower types of glycerid although the diglycerid is probably still the main product.

The pancreatic lipase, although secreted with the pancreatic juice in water-soluble form, is with difficulty extracted from the gland by water. Glycerol is generally used for the purpose and the result is a suspension which may become inactive on filtration, indicating that the lipase is probably not in true solution.

Pancreatic lipase is secreted mainly in the active form, and its activity is increased by the presence of bile (bile salts) and by many other substances as, for example, blood serum, soaps, saponins, alcohol, etc. Its action is inhibited by cholesterol. Rosenheim has succeeded in separating from the lipase of pancreatic extracts (glycerol) a co-enzyme without which the enzyme is inactive. As is generally the case with co-enzymes this one is heat-stable. Since the inactive enzyme is activated by blood serum the assumption is made that the activating substance is a hormone produced by the pancreas and secreted into the blood.

Normally the provisions for the digestion of the fats in the intestine are such as to insure practically complete splitting. Fat is delivered to the intestine in small amounts—when there is little fat in the food this follows as a matter of course; when fat is present in large proportion emptying of the stomach is slowed, whereby the same result is effected. Lipase is abundant, being found both in the gastric secretion and in the pancreatic and intestinal secretions. The amount in the pancreatic secretion alone is sufficient to digest quickly several times the amount of fat supplied in the ordinary diet. The gastric lipase, under favorable conditions, can digest considerable quantities, and even the intestinal lipase can probably affect splitting of the daily quota of fat, since in cases where the pancreatic secretion is lacking very little unsplit fat is found in the feces. Emulsification by soap is an important factor in the hydrolysis, and there is normally abundant provision for the formation of soap. There is always some free fatty acid in natural fats, and the amount is increased by cooking and by the action of the gastric lipase,

so that by the time the fat reaches the intestine a considerable amount of free fatty acid is present. The free fatty acid is neutralized by the alkali carbonates of the various secretions that find their way into the intestine, forming soaps which quickly and completely emulsify the remaining fat, thus preparing it for rapid digestion by the lipases. Added to the other factors is the continuous absorption which removes the products of hydrolysis from the field of action, thereby in a balanced reaction like the hydrolysis of a fat, providing the best conditions for rapid and complete action. Under these conditions it is probable that the amount of fat which escapes digestion is negligibly small.

The Absorption of Fat from the Intestine.—The manner in which the fat leaves the intestine has received its share of experimentation and speculation. The earlier belief was that the fat was absorbed as such in emulsified form, based largely on the observation that emulsions are often found in the intestine during fat absorption and that the fat in the chyle is also in the emulsified form. While it was known that the chyle fat was in general much more finely divided than the intestinal fat, that objection might be explained away by the assumption that the particles were absorbed only as they reached a fine state of division. Further evidence believed to point in the same direction is that large amounts of characteristic food fat may be laid down in the fat depots of animals with slight change. Another argument, later shown to be faulty, was that if a stained fat were fed similarly stained fat appeared in the chyle. An additional bit of evidence in favor of absorption of unchanged fat was the observation of Ravenel that bacteria may be carried through the intestinal wall if fat is fed along with them when they do not pass through otherwise. The fact that other foodstuffs such as the carbohydrates and proteins were known to be absorbed in water soluble form and that much free fatty acid and soap were to be found in the intestine during fat digestion led Kühne to put forward the hypothesis that fats also were absorbed in water soluble form, being first split in the intestine and then resynthesized in passing through the intestinal wall. This hypothesis brought forth a large amount of experimental work which finally resulted in practical adoption as the most satisfactory explanation of the method of transference of fat from the intestine to the blood.

The earliest conclusive work on the subject was that presented by Munk (*a*) (1891), who, making use of a human patient with a chyle fistula, was able to show that fatty acids and esters of the fatty acids with alcohols other than glycerol were absorbed, appearing in the chyle not as these substances but as neutral triglycerids. He was followed by v. Walther, who confirmed his results with fatty acids or soaps, and more recently by Frank (*c*) (1898), with ethyl esters of the fatty acids and Bloor (*a*) (1913) with an optically active mannite ester of a fatty acid. In all these cases the evidence indicated that no trace of the substance fed appeared in

the chyle but always the glycerol triesters of the fatty acids involved. The presence of the glycerids in the chyle presupposed a splitting of the esters fed and a synthesis of the fatty acids with glycerol which if not supplied with the fatty acids by the experimenter must have been furnished by the organism. Further details on this interesting point have been furnished in recent work by Bang (*a*) (1918), who found that fatty acids alone produced but little lipemia while when these are fed with glycerol there is marked lipemia, indicating that the ability of the organism to supply glycerol is limited. One experiment which he reported in which he fed 59 grams of fatty acid to a dog and recovered only 2 grams in the chyle would indicate that absorption in this case was directly into the blood.

Direct evidence against the absorption of fat in emulsified form has also been forthcoming. Connstein, experimenting with lanolin, a wax which emulsifies well with water and has a melting point (40°-42° C.) only slightly above body temperature, showed that when this substance was fed about 98 per cent of it could be recovered in the feces, showing that neither emulsifying power nor melting point was the criterion for absorption. The same fact was more strikingly shown by Henriques and Hansen, who dissolved vaselin in lard and fed the well emulsified mixture to rats and were able to recover practically all (98 per cent) of the vaselin fed while the lard was completely absorbed. The companion test to this one—the attempt to recover the substance from the chyle—was carried out by Bloor (*a*) (1913) with negative results. In this experiment a liquid paraffin was dissolved in olive oil, the whole well emulsified and fed to dogs. A suitable time after the feeding chyle was collected from the thoracic duct, the contained fat extracted and examined for the paraffin oil. None was found. Thus though all conditions were favorable for the absorption of unchanged emulsion which would have included the mineral oil, no trace of it could be demonstrated while the food fat was completely absorbed. Summing up all the evidence then, the hypothesis of Kühne appears to be very well supported. Facilities are provided for complete splitting of the fats in the intestine, fatty acids and soaps are absorbed and appear in the chyle as triglycerids, esters of the fatty acids which are hydrolyzable by the intestinal lipases are absorbed but always as triglycerids, while non-hydrolyzable esters of the fatty acids and other fat-like substances which cannot be made water soluble are rejected. Altogether it seems likely that fats are no exception to the rule that substances pass from the intestine only in water solution, and since solubility in water appears to be a necessary prerequisite for use in living cells the intestine acts as a barrier against the admission of substances that cannot be made soluble. The fact that fats appear in the blood stream largely in the insoluble suspended form is probably only an apparent exception since they are readily and quickly transformed in the blood into soluble phospholipoid.

Synthesis of the Fats During Absorption.—It is a necessary corollary of the foregoing that the splitting of the fats which takes place in the intestine is followed by a resynthesis before the fat reaches the thoracic duct. Direct proof of the synthesis has, however, not been satisfactorily furnished. Ewald thought that he had demonstrated a synthesis by the surviving intestinal mucous membrane, as did also Hamburger, but Frank and Ritter, on repetition of their experiments, were unable to get positive results, and pointed out that their results were irregular and that such positive findings as were obtained were due to faulty technique. Similarly Moore failed to demonstrate synthesis in vitro using mixtures of sodium oleate and glycerol with hashed intestinal mucous membrane. On the other hand, Moore showed that during fat absorption the fatty acid in the mucous membrane of the intestine amounted to 15-35 per cent of the total fat, while in the mesenterial glands and lymph vessels it amounted to only about 4 per cent, which facts they believed to show that the synthesis took place in the mucous membrane and not in the lymph glands.

Paths of Absorption of Fat.—The thoracic duct is probably not the only channel by which fat reaches the blood stream. Munk and Rosenstein in chyle fistula experiments with a human being were able to recover not more than 60 per cent of the total fat fed. In experiments with dogs Munk and Friedenthal were able to show an absorption of 32 to 48 per cent of the fat fed after tying off all the neck and arm veins of both sides. The blood fat increased from 0.5 per cent to 2.92 per cent, with notable increases of fat in the corpuscles. Others have found, on the contrary, that tying off the thoracic duct prevented any increase in blood fat. Munk also noted the accumulation of fat droplets in the liver during normal fat absorption ("physiological fat infiltration"), which he believed to originate from fat directly absorbed into the portal vein—although it could equally well be ascribed to fat which had reached the blood stream by the thoracic duct. v. Walther found in the chyle not more than 1/10 of the fat which had disappeared from the intestine of dogs. A similar observation is reported by Frank (1898). Attention should be directed to the fact that in these thoracic duct experiments the operative procedure is severe and the results found may not represent what happens normally. Aside from the thoracic duct there is left the path of absorption taken by other foodstuffs, i. e., directly into the circulation by the intestinal capillaries and the portal vein, but there is very little direct evidence of absorption by this channel. D'Errico showed that during fat absorption the fat content of the portal vein was always higher than of the jugular and concluded that fat was normally absorbed directly into the circulation like other food substances. Very recently Zucker has reported negatively on repetition of this work.

Changes in Fats During Absorption.—In spite of the fact that large

amounts of food fat may by certain treatment be transported without considerable change directly to the fat depots, evidence is available to show that under normal conditions where the animal has free choice of food and where the amount of fat ingested is not too large, the fat in the chyle may be noticeably different from the fat in the intestine. Two factors appear to be at work in the production of the differences: (a) selection from the food fat of the more desirable or useful portions (generally the lower melting), and (b) other changes either of the nature of additions or of chemical changes—saturation or desaturation—which may alter the composition considerably. With regard to the first factor—selection—Munk has found that in dogs fed with lard the feces fat had a considerably higher melting point than the fat fed. With regard to the second factor—admixture or alteration—during the passage from the intestine, Munk and Rosenstein after feeding cetyl palmitate found the chyle fat to consist of one part of triolein and six parts tripalmitin, with a melting point of 36° C. Frank (1898), after feeding ethyl palmitate, found 36 per cent of olein in the chyle fat, and after feeding mutton tallow (m.p. 51.7° C.) obtained a chyle fat melting at 38° C. In these cases there was an alteration in the direction of obtaining a lower melting fat. Bloor (1913-14) obtained evidence of an alteration in the other direction, i. e., the chyle fat having a higher melting point than the fat fed. After feeding olive oil of which the constituent fatty acids had a melting point of 16° C. and an iodin number of 86, chyle fat was obtained with a melting point of 30° C. and iodin numbers down to 72. Other evidence corroborating the above findings was furnished by Raper (1912-13). In most of these cases the influence of lipoids present in the fasting chyle was excluded so that we may conclude that the fat may be considerably modified during the process of absorption. The modifications as found appear to be purposive in that in all cases the tendency appears to be toward the production in the chyle of a fat approximating the properties of the body fat of the animal. As to the significance of these changes Frank was of the opinion that there is an addition of body fat either by way of secretion into the intestine or after the fat leaves the intestine. It has been shown by Leathes (1909) that the liver has probably the power of desaturating the fatty acids—a power which all living cells may possess to some degree, and there is a possibility that the intestinal cells can desaturate or saturate the fatty acids during their passage through. The mechanism would allow adaptive changes in the fats during absorption.

Factors in Fat Digestion and Absorption.—*Pancreatic Secretion.*—The pancreas is the main source of lipase in the intestine. The amount of secretion, generally given at 500 to 600 c.c., is sufficient for the rapid hydrolysis of at least its own weight of emulsified fat, and since the amount of fat in the daily human diet does not often exceed 100 grams, is greatly in excess of the needs. In the absence of pancreatic secretion,

the amount of fat absorbed falls off, but not to the extent that would be expected from the loss of such an important secretion. Also, as has been noted many times, the fat which is found in the feces in these cases is almost entirely present as fatty acids, indicating that the other hydrolytic agents present (see previous discussion, pages 189-192) and also probably bacteria very effectively take on the work of the pancreatic lipase. Complete extirpation of the gland produces much more marked effects than exclusion of the secretion. Emulsified fats are better utilized than non-emulsified and feeding of pancreas improves the utilization of both. With regard to complete extirpation various factors complicate the situation, such as shock of operation, deprivation of the internal secretion, both of which are severe in their effects on the animals, the inability to digest and utilize other foodstuffs, which results secondarily in a failure to utilize fat, the efficiency of the pancreatic secretion in forming emulsions which are stable in the faint acidity found in the intestine, the disturbance in the intermediary metabolism of fat which results in an accumulation of fat in the liver and other tissues and the slowing of the emptying of the stomach in the absence of pancreatic secretion.

Taking all the evidence together there can be no question that the intestinal secretion of the pancreas is an indispensable factor in the proper digestion and absorption of fat. Whether its internal secretion is of equal importance cannot be stated at the present time. Lombroso found that fat absorption was not much affected by stopping the pancreatic secretion or on extirpation, if a small portion of the gland were left in place, from which he reasons that it is the internal secretion which is of importance. On the other hand, it is well known that in severe diabetes where the carbohydrate tolerance is very low, that fats are readily digested and absorbed, and indeed in such amounts that they cannot be taken care of in the blood, resulting in the extreme and lasting lipemia which is occasionally reported. The lipemia may be the direct result of the absence of internal secretion, resulting in failure of the intermediary fat metabolism or a secondary effect of the failure to utilize carbohydrate.

The Bile.—The importance of the bile in the digestion of the fats has been extensively studied. Early experiments by Claude Bernard and Dastre demonstrated the probable necessity of both bile and pancreatic secretion for effective fat absorption. Work by Bidder and Schmidt, Röhmann and others have shown that exclusion of the bile from the intestine may result in fat losses up to 85 per cent of the fat fed. In icterus with complete exclusion of bile there is considerable loss of fat, but not to the extent observed in operative exclusion. The importance of bile in fat absorption seems thus to be well established. As to its function in this relation evidence has been brought forward by Moore and Rockwood to show that one very important part which it plays is in increasing the solubility of the fatty acids and soaps produced by hydrolysis of the

fats. It also increases the formation of soaps from the fatty acids as shown by Pflüger, and later by Kingsbury. These effects are partly due to the bile salts but to a considerable extent to other substances, e. g., mucin and lecithin.

The accelerating or activating effect of bile on the pancreatic lipase has been shown by Rachford and by von Fürth and Schütz, who found that the fat splitting power of pancreatic juice was increased several fold by the presence of bile. The active substance in the bile which produces the acceleration has been shown by both investigators to be the bile salts. Aside from any positive action of the bile the mere exclusion from the intestine of a pint or more of alkaline colloidal secretion must have a profound effect on intestinal processes. As regards further and unknown functions of the bile mention should be made of the important findings of Hooper and Whipple that dogs cannot long survive complete exclusion of bile from the intestine unless liver is included in their diet.

In the absence of both bile and pancreatic secretion very little fat is absorbed, probably not over 20 per cent of emulsified fat, is in milk, and much less of non-emulsified fat, although splitting is generally good—80 to 90 per cent of the rejected fat consisting of free fatty acids. Traces only of soaps are present, which would point to the lack of alkali ordinarily furnished by the pancreatic secretion and the bile as the significant factor in absorption.

The Nature of the Food Fat.—Lipase can act only on the surface of the fat, hence the necessity as a preliminary step, of breaking up the fat masses to as fine a state of division as possible as in emulsions, so as to increase the available surface. For ready emulsification the fat must be liquid or at least semi-solid at body temperature, and we find that the utilization of a food fat depends largely on its fluidity at body temperature. Thus v. Walther, in feeding experiments, found that various fats which were liquid at body temperatures were absorbed to the extent of 97 to 98 per cent, while tristearin (m.p. 60° C.) was absorbed to the extent of only 14 per cent. Dissolving tristearin in almond oil so as to bring the melting point down to 55° increased its absorption to 89 per cent, indicating the importance of the liquid fats and especially of triolein as a solvent for the harder fats, making it possible to deal with them in the organism both in hydrolysis and in transport. On the other hand, experiments with ethyl stearate (m.p. 30° C.) have shown that melting point in the intestine is not the only factor in absorption, since this substance is very little better absorbed than tristearin, although it is liquid at body temperature. Also when it reaches the thoracic duct (as tristearin) it was found mixed with enough softer fat to bring its melting point down to near body temperature. It seems from these experiments that the organism is able to protect itself against the absorption of high melting fat which it would have difficulty in dealing with, first by limiting the

amount absorbed and second by mixing it with enough low melting fat to bring the melting point of the mixture to somewhere near body temperature. (Recent work by Lyman indicates that available glycerol may be a limiting factor in absorption of the simple esters, just as it is with the fatty acids.)

Aside from the high melting fats and excepting certain ones like castor oil which are either irritating to the intestine or which form irritating soaps, there appears to be little difference in the extent of utilization of fats of whatever origin, animal or vegetable, a result which might have been foretold since the fatty acids in combination in fats from various sources are largely the same, the main difference being in the relative amounts of each constituent of the mixture.

Emulsification in Fat Digestion and Absorption.—It is generally assumed that fats must be emulsified in the intestine before they can be digested and absorbed, for the reason that while the lipases found in the intestinal secretions are always in water solution the fats are insoluble in water and lipolysis can take place only at the surface, which emulsification greatly increases. The assumption has the support of a large number of observations on fat in the intestine during digestion. That emulsions are not always present in the intestine under these conditions is, however, attested by observations of Moore and Rockwood, who found in many cases no emulsion but a brownish liquid with an acid reaction. No examination was made as to whether this liquid contained fat and it is possible that it consisted of a bile solution of the fatty acids. Where conditions for digestion are exceptionally good the emulsion may be only transitory. The conditions for the emulsification of the food fat on its entry into the intestine are ordinarily very favorable. There are present free fatty acid in the fat, alkali in the secretions, and other substances such as proteins, lecithin, etc., which are either emulsifiers themselves or which act to stabilize emulsions. The acidity of the intestine which many observers have found need not be a hindrance since it is due mainly to carbonic acid and emulsions formed with the aid of pancreatic secretion and bile are known to be stable in solutions of carbonic acid.

Summary.—It will be seen that no definite answer can yet be given as to the way in which fat passes through the intestinal wall. Emulsification is probably at least an early if temporary step. Hydrolysis undoubtedly takes place in large measure and would therefore seem to be a necessary preliminary to absorption. Soap formation under the conditions of reactions of the intestinal contents (faint acidity) and the presence of bile probably takes place to a considerable extent. Soap being water-soluble is assumed by many to be the form in which the fats are finally absorbed, but it should be borne in mind first that soap is a difficultly diffusible substance and second that in water solution it hydrolyzes, forming aggregates of free fatty acid which would be still

less diffusible. On the other hand, the earlier theory of absorption of fat as such has secured some additional support from the observations of Green and Skaer that fats can penetrate for considerable distances into the stomach walls of animals, confirming on animals the much earlier observation of Schmidt that fat penetrates readily into plant cells, especially if it contain a little free fatty acid. The ability of certain types of animal tissue cells to engulf foreign particles, including fats, has been shown by Evans, just as the phagocytic white blood cells are known to do. (The part which these same white blood cells take in fat absorption, while known to be large for the individual cell, is not believed to be important in the aggregate.) However, even in plants a preliminary hydrolysis would seem to be necessary since in fat seeds, such as the castor bean, hydrolysis is known to take place before the fat is utilized. Even so, hydrolysis produces another kind of insoluble substance—the fatty acid—which, however, is different and probably essentially so in that in the presence of alkali it becomes water soluble. To what extent fat passes the intestinal walls as fatty acid—bile being the ferry, as has been suggested by Mathews—cannot be determined. Neither can it be said what factors determine whether the digested fat shall pass directly into the blood by way of the portal system or indirectly by way of the thoracic duct. In the former case it passes directly to the liver, and in the latter it avoids it. It seems quite certain that esters of the fatty acids which cannot be hydrolyzed in the intestine and so rendered water-soluble and also oily substances of other kinds which cannot be made water-soluble are rejected no matter what their other properties may be nor how intimately they may be mixed with the fats. Water solubility of the absorbed products seems to be as essential for the fats as for other food substances. The mechanism for excluding substances which are not water-soluble is perfect, presumably because such substances could not possibly be handled in the organism.

Fat in the Blood

Alimentary Lipemia.—The study of the blood brings us one step nearer to the actual seats of metabolism than that of the urine and other waste products. It is the great distributing system of the body. The recognition of these facts has turned the attention of most investigators to the blood, with the result that thereby much has been added to our knowledge of metabolism. Because of the greater difficulty of their study the discoveries regarding the fats have as usual rather lagged behind those of the other foodstuffs, although a good deal has been accomplished. Methods for fat determination in foods and tissues have been adapted for use with blood, and new methods have been devised especially suited to use with small amounts of blood, so that processes can be followed in

the living animal with considerable exactness. The result has been an accumulation of data from which we can now begin to get an insight into the history of the fats after they leave the intestine. After absorption that part of the food fat which has passed into the lacteals finds its way into the blood stream by way of the thoracic duct in the form of a suspension of very fine particles (generally less than 1 μ in diameter), in which the Brownian movement is marked and which give the chyle and the blood plasma their milky appearance. The milkiness persists for some time but has generally disappeared in from eight to fourteen hours after the fat is eaten. According to present observations milkiness persisting fourteen hours after a meal indicates an abnormality in fat metabolism. Emulsified fat (particles 2 to 5 μ in diameter) injected directly into the veins disappears within a few minutes, the difference from alimentary lipemia being due probably to the larger size of the fat particles, although there is a possibility that the relatively small amount injected would be quickly removed and stored while a larger amount would not. Rabbeno found that homogenized fat (particles up to 2 μ in size) injected in quantity disappeared rather slowly (7 hours). The extent and duration of the increase of the blood fat following a meal depends on the amount of fat fed and also apparently on the level of the blood lipoids at the time of feeding. When the blood lipoid level is high the maximum in the blood is reached sooner and the fall from the maximum is slower than is the case when the lipoid level in the blood is low. The amount of extra fat in the blood does not, however, at any time represent the amount which has disappeared from the intestine so that absorption by the tissues from the blood must normally be rapid. The extent of alimentary lipemia varies greatly in different animals. In rabbits it is very difficult if not impossible to produce. In geese stuffed with rye values as high as 6 per cent have been recorded. This is probably a cumulative value, since under these conditions fat absorption must be continuous. In dogs the blood fat values rarely exceed three per cent, and in humans two per cent. In human beings with diabetes, lipemia, which is probably primarily of alimentary origin, with values of over 20 per cent, has been recorded, and while this is an extreme instance, high values are not uncommon in untreated cases. The passage of fat from the blood is probably inhibited in these cases, since on a low calorie low fat diet it may take a month for values to get down to normal.

The mechanism of the disappearance of fat from the blood is uncertain. Stained or otherwise distinguishable fat injected into the circulation disappears promptly as indicated, and is found to have accumulated in the liver, bone marrow, spleen and muscles in the order named—which is true also of other finely suspended material of other kinds. During fat digestion the fine fat particles are found to have accumulated in various places along the endothelial lining of the blood vessels. Various theories have

been advanced to explain the way in which the material passes across the vessel walls into the tissues. One of the earliest was that there is the same process of hydrolysis and resynthesis as takes place in the passage of the intestinal wall, which postulates the presence of lipases in the neighborhood of where the transfer takes place. In this connection much confusion has resulted from the failure to distinguish between "esterases"—enzymes which can hydrolyze simple esters such as ethyl butyrate and also, though more slowly, glycerids of the lower fatty acids, as for example tributyrin, but cannot hydrolyze ordinary fats (or, at least, only very slowly), and true lipases such as are found in the pancreatic secretion, which split fats readily; and still further uncertainty has been caused by the failure to exclude cells or portions of cells from the extracts used for testing. Esterases appear to be quite widespread in the blood and tissues, although generally in small amounts and of slight activity, while lipases in significant amounts appear to be confined to the pancreas. Even in the mammary gland and the fat depots where the exchange of fat would presumably be most active no significant amount of lipase can be demonstrated. So that the primary requisite for hydrolysis and resynthesis, an adequate supply of lipase at the tissue cell wall is missing. On the other hand, esterases which are capable of splitting lecithin are found to be quite widely distributed (Thiele, 1912-13) and, for reasons which will appear later in the discussion, are believed to be of importance in fat metabolism.

Coincident with or immediately following the rise of fat in the blood during fat absorption certain changes have been noted in the other blood lipoids which appear to be of importance in fat metabolism. A considerable increase of lecithin is noted by all workers. A similar increase of cholesterol is found by some but not by others, which may be explained by the fact that it apparently comes later. It is becoming more and more evident that these three substances—fat, cholesterol and lecithin—are closely connected in fat metabolism, and when one is increased the others are very generally also found to be similarly high. The period during which fat is abnormally high in the blood during fat absorption (about eight hours) is apparently long enough to produce increases of lecithin, which follow quickly the increases in fat, but may not be long enough to bring about increases of cholesterol which take place later and more slowly. The close relation of lecithin and cholesterol to fat would indicate that these are stages in metabolism through which the fats may or must pass before they are utilized, a supposition which is supported in the case of lecithin by the close similarity in composition and in the case of cholesterol by the constant relation in the blood serum between cholesterol and its fatty acid esters.

The blood corpuscles appear to take a considerable part in the changes in the blood lipoids during alimentary lipemia. The old observation of

Munk and Friedenthal that the fat content of the corpuscles increased during fat absorption has been recently confirmed and it was also shown that the increase of fat was accompanied by increases of lecithin, from which the inference was drawn that the corpuscles take up the suspended fat from the plasma and transform it into lecithin. Some support is given to this inference by the observations of Thiele and of Foà (1915), who found that the blood esterase decomposes lecithin only when corpuscles are present, indicating that this esterase, which presumably also synthesizes lecithin, is present only in the corpuscles. On the other hand, later work in this laboratory has shown that in certain dogs lecithin does not markedly increase in the corpuscles but does in the plasma. As has been recently pointed out by Bang (1918), animals show great individuality in their blood reaction to ingested fat. Some can dispose of large amounts without showing much effect on the blood lipoids; others react strongly. He makes some suggestions to explain the differences—habituation to fat food and the presence of carbohydrate in the food or of much stored glycogen being in his opinion important factors. As regards lecithin formation in the blood it is not likely that it is confined to the corpuscles but probable that other cells with which the suspended fat comes in contact have the same function. Furthermore, the failure to find increased lecithin values in the corpuscles of certain animals does not necessarily mean that it is not formed there. It may be formed and pass at once into the plasma.

Lipoids of the Blood.—A great deal of investigative work has been done on the lipoids of the blood both in the normal and in various pathological conditions, the results of which in general bear out the rule just enunciated, that when one of the constituents (fat, cholesterol, lecithin), is found abnormal the other two will also be abnormal and in the same direction. It has been shown how feeding fat increases the blood lecithin, and while there is some question as to whether blood cholesterol is increased in the lipemia produced by a single fat feeding there is none at all where the lipemia persists. Feeding cholesterol produces not only increase of blood cholesterol but also of blood lecithin. Whether feeding lecithin would produce increases in the other two constituents has not been reported and probably cannot be determined since lecithin is largely hydrolyzed in the alimentary tract and probably absorbed as fat although some may appear as such in the chyle. While there are not enough data available to justify the statement that there is a constant relation between the three constituents in normal and in most pathological conditions, the tendency seems to be in that direction and, at any rate, it appears reasonably certain that the three substances are interdependent, and also that all are concerned in the metabolism of the fatty acids.

The concentration of fat, cholesterol and lecithin in the blood is fairly constant for the same species but varies greatly in different species, the variation being noticeable mainly in the plasma. The concentration in

the plasma and the corpuscles of the same animal is different. In general, the lipoid level in the plasma is higher in the carnivora than in the herbivora, being undoubtedly influenced by the amount of fat habitually present in the diet. There is no such difference between the concentration of the lipoid constituents in the corpuscles of the various species, the tendency being rather to a similarity of composition in all.

The level of the blood lipoids may be affected by various conditions, the most frequent being alimentary lipemia as discussed above. Other foods than fat apparently do not affect the level, at least not unless the diet is continued for some time. Fasting for short periods may or may not raise the level of the blood lipoids (dogs), depending probably on the nutritional condition of the animal. After the first two weeks of fasting there is generally a slow fall, although here again the nutritional condition of the animal at the beginning of the fast is probably important. Narcotics—chloroform, ether and alcohol (especially the two latter)—if long continued generally cause an increase of the blood lipoids. Chloroform may not produce any effect during or immediately after the narcosis, but the effects may appear two or three days later. As reasons for the effects may be given the increase in the lipoid solvent power of the blood due to the dissolved narcotics and also their poisonous effects on the tissues, especially the fatty tissues—which absorb these substances selectively—producing more or less disintegration of the cells. Poisoning with phosphorus or phlorizin will sometimes produce an increase of the blood lipoids, but the reaction is not constant. In late pregnancy in mammals there is often a rise in blood lipoids, due probably to preparation for lactation. It has been found that there is a relation between the level of blood lipoids and the amount of fat secreted in the milk of lactating animals, also that the lipoid phosphorus is higher in lactating animals than in dry ones.

Fat in the Tissues

Storing of Fat.—Lipoid material exists in the tissues in two states or conditions: (a) stored, or inactive, consisting of almost pure fat with not more than traces of other lipoids; and (b) cell lipoid, "built in" or active, forming part of the living tissue and taking an active part in life processes. Of this latter, phospholipoid is the one present in largest amount and widest distribution, then cholesterol and its compounds followed by the series of more or less well characterized substances which include most of the known lipoids. The cell lipoids are relatively constant in composition and appear to be characteristic of the tissue.

Stored fat is found in various parts of the animal body, mainly in more or less well defined fat depots such as the abdominal, subcutaneous and intermuscular, and around the organs. It is not normally found

in more than small amounts in active tissues such as the heart, kidney and muscles, although considerable lipoid material of other kinds is present there. The stored fat has its origin in part directly from the fat of the food and in part indirectly by synthesis from other food substances, mainly carbohydrate. Synthesis from protein probably does not ordinarily take place to any considerable extent. Under certain circumstances—stuffing of an animal with fat, especially after starvation—food fat may be laid down in the fat depots with but little if any change, but under ordinary conditions where the animal has a normal choice of food there is a marked tendency to produce a fat characteristic of the animal; for example, beef fat has certain definite characteristics which distinguish it from hog fat and both from human fat. The laying down of a characteristic body fat by an animal from its food must involve several factors such as choice from the food fat as to which portion is to be immediately consumed and which stored, the nature of the fat synthesized from carbohydrate, also, in case the stored fat is used, choice as to whether the harder or softer constituents are to be used first, since there is some evidence to show that the fat of a starved animal has a higher melting point than the normal body fat of the animal. Although the laying up of a characteristic fat is partly the resultant of these factors, their activity is limited and in the end the fat stored is greatly influenced by the food fat especially if it forms a large proportion of the diet. The question has a considerable economic interest in connection with the fattening of animals, e. g., hogs for market, since it has been found that if too much liquid fat is included in the diet the result is a soft meat from which the fat oozes out on standing.

Changes in Fat in the Tissues.—If the stored fat is thus markedly influenced by the food fat, the built in fat or cell lipoid is just as notably characteristic of the tissue and uninfluenced by the food fat, and since the fatty acids found in combination in the cell lipoids are often different from those ordinarily found in the food, the question arises as to the power of the tissues to alter for various purposes the fat presented to them. The differences between the fatty acids of the active tissues and those of the food consist mainly in (a) their degree of saturation, (b) the groups with which they are combined. They are in general much more unsaturated, the iodin absorption value of the fatty acids of the tissues is found to be in the neighborhood of 130, while that of the stored fat is from 35 to 70. The iodin value of the blood lipoids in normal human beings is about 66 (calculated). The fatty acids in the tissue cells are largely combined as phospholipoids, although there are also a number of other combinations of the fatty acids to be found in the organs and in the brain and nervous tissue. These, with few exceptions, are not well understood chemically, and since they apparently take but a small part in ordinary fat metabolism they will not be considered here. The presence of compounds of the unsaturated fatty acids, especially phospholipoids, in

large amount (up to 15 per cent) in the cells of continuously active organs like the heart and kidney as well as in lesser percentages in the muscles furnish a basis for the theory that they constitute the form in which the fats are utilized, and that food fat must undergo these changes—desaturation and phosphorization—before it can be utilized. The theory is given support from the fact already discussed that whenever there occurs a large accumulation of fat in the blood, most frequently in alimentary lipemia, there is accompanying it a marked increase in the amount of lipoid phosphorus present.

The Liver in Fat Metabolism.—That the liver plays an important part in fat metabolism is indicated by the work of many investigators. Munk (1902) found that the liver was loaded with fat during fat absorption. Leathes and Meyer-Wedell, by the use of a fat with high iodin number, found not only that the accumulated fat of the liver after feeding was food fat but that the liver was the only organ in which such marked accumulation occurred. In various abnormal conditions, such as poisoning with phosphorus, chloroform or phloridzin, in diabetes, in starvation, etc., great increases of the fat in the liver may occur which are believed to be the result of mobilization of stored fat since the fat found in the liver at these times has the properties of stored fat rather than of normal liver fat.

The accumulations of fat in the liver whenever fat is being extensively moved by the blood stream indicate that the liver must have an important function in fat metabolism. Is it a temporary storehouse by means of which the fat in the blood is kept within limits as is the case with the carbohydrate, or does the fat undergo some essential change there? Leathes' theory of the function of the liver in fat metabolism is that mobilization of fat to the liver is a normal process, that the fat is brought there for two purposes: (a) introduction of double bonds (desaturation) which paves the way for breaking the long fatty acid chains into shorter ones, and (b) phosphorization of the fat, changing it into phospholipoids which increasing evidence seems to show is the initial stage in the intermediary metabolism of fat. The desaturation he believes to be specific for the liver, but phosphorization may be accomplished in other places. His theory is based on the following evidence: The fatty acids ordinarily found in the liver differ from those of the stored fat in being much more unsaturated. The liver is the only point of mobilization of fat from the intestine or the fat stores. The inference is that the liver desaturates the fatty acids which are brought to it. Since, however, similar unsaturated fatty acids are found in other organs like the heart and kidney it might with equal correctness be inferred that desaturation occurs in these also. Some work by Mottram with the plaice in which he found that the fatty acids of the liver had a lower iodin number than those of either the food or the muscles, would indicate that the liver may not always have the function of desaturation. But as it is the only place where

temporary accumulations of fat occur and is the most important gland in the organism the probable correctness of Leathes' hypothesis as regards desaturation must be admitted. That phosphorization takes place in other locations than the liver is indicated by work on changes in fat in the blood in which it is shown that the blood cells may have this function. Allowing the correctness of the assumption that phospholipoid ("lecithin") is the essential intermediate step in fat metabolism, the questions of fat transport in the blood and in and out across cell walls after it enters the blood stream as well as its further utilization are greatly simplified, since lecithin is soluble in the blood plasma and since there are present in all organs and tissues esterases which hydrolyze lecithin readily but which have little effect on the fats. That blood lecithin may be a source of fat in the living organism is well shown by the work of Meigs and coworkers, who found that milk fat could be satisfactorily accounted for by decreases in lecithin in the blood passing through the mammary gland.

Later Stages—β-oxidation.—As regards later stages in the intermediary metabolism of the fats little is definitely known. The fatty acids ordinarily disappear in metabolism without leaving any traces in the way of intermediate stages by which the process of breakdown may be followed. In certain cases, however, as in severe diabetes and even in short periods of fasting, acids appear in the urine which are now believed to be late stages of fatty acid combustion. These unburned residues are β-oxybutyric and diacetic acids which with their derivative acetone constitute the "acetone bodies." That these substances are actually stages in the breakdown of the fatty acids is strongly indicated by the work of Knoop, whose hypothesis of β-oxidation seems to account satisfactorily for the final stages in the process of oxidation and breakdown of the fatty acids. For the stages between we can only surmise. As pointed out by Leathes the introduction of double bonds produces points of weakness in the long chains where oxidation with subsequent breaking readily takes place, producing shorter chain mono- and dicarboxy acids. (In this connection it is interesting to note that in such a process of oxidation and breaking down, only one monocarboxy acid would be produced from a long chain fatty acid, the other fragments being dicarboxy acids. Thus from an unsaturated fatty acid of the linoleic series such as Hartley finds in the liver,

$$CH_3 \cdot (CH_2)_4 \cdot \overset{H}{C} = \overset{H}{C} \cdot CH_2 \cdot \overset{H}{C} = \overset{H}{C} \cdot (CH_2)_7 \cdot COOH$$

there would be formed,

$CH_3 \cdot (CH_2)_4 \cdot COOH$ caproic acid $\quad CH_2 \cdot (COOH)_2$ malonic acid and $(CH_2)_7 \cdot (COOH)_2$ azelaic acid

of which the dicarboxy acids would presumably have a different type of metabolism from the monocarboxy acids.)

Knoop's hypothesis that the fatty acid chains are broken down two carbon atoms at a time is supported by the following evidence (Knoop, F. (*a*) 1904-05. Making use of benzol derivatives of the fatty acids which are utilized with much more difficulty in the organism than the fatty acids themselves, he found that the fatty acid side chains on the benzol nucleus are broken down two carbon atoms at a time and that the breaking is preceded by oxidation at the β-carbon atom. Oxidation of the fatty acids in vitro usually takes place at the α-carbon atom, and Knoop's theory was received skeptically by chemists until further work by Dakin confirmed his results both on animals and in vitro, and indicated that β-oxidation is probably the common type of oxidation of the fatty acids in the animal organism. The theory adequately explains the appearance, in diabetes and other conditions, of β-oxybutyric and its derivatives, which are regarded mainly as residues of the fatty acids which have escaped complete combustion because of an abnormality in metabolism. Later work has shown that certain groups in the protein molecule may also form "acetone bodies," but it is believed that this source is relatively unimportant.

The fact that the fatty acids are broken down two atoms at a time and the fact that naturally occurring fatty acids contain even numbers of carbon atoms would render it probable that they were built up two carbon atoms at a time, affording a basis for a theory of fatty acid synthesis from carbohydrates in support of which there is considerable experimental evidence. That fat is formed from carbohydrate has long been known empirically since farm animals are ordinarily fattened on a diet which consists mainly of starch; and scientifically acceptable proof was furnished by Lawes and Gilbert many years ago. The probable mechanism of the synthesis has been indicated by changes which take place readily in carbohydrates. Thus sugars readily yield lactic acid by various treatment—action of bacteria, of weak alkalies, etc., and lactic acid in turn breaks down readily to acetaldehyd. The acetic aldehyd by aldol condensation may be made to form β-hydroxybutyric aldehyd, which by shifting of the oxygen atom—simultaneous oxidation and reduction—yields butyric acid. The butyric acid fermentation of dextrose or lactic acid observed by Pasteur may probably be explained in this way. The likelihood of this procedure being the true method of synthesis of the fatty acids is rendered probable by the work of Raper (1906-07), who showed that in addition to butyric acid, caproic and caprylic acids are formed, and that the synthesis of higher fatty acids may be brought about *in vitro* from aldol and therefore from acetaldehyd. Smedley has raised objections to the assumption that the higher fatty acids are formed from acetaldehyd by aldol condensation, basing her objection on the fact that the aldol condensation when applied to the higher aldehyds *in vitro* produces branched chains instead of straight chains, also that no free aldehyds (except sugars) are found in the living organism. She suggests as the

probable intermediate stage between carbohydrate and fatty acid, pyruvic acid $CH_3.CO.COOH$, which she has shown to produce straight chain higher fatty acids *in vitro* by condensation with fatty aldehyds. To get around her own objection that aldehyds are not found in living organisms she postulates that combination is affected with aldehyds in the "nascent" condition. The earlier suggestion of Emil Fischer that the higher fatty acids are formed by direct condensation of sugar molecules with reduction and oxidation has neither chemical nor biological evidence to support it, but is nevertheless interesting since the most widely distributed fatty acids, stearic, oleic, linoleic, etc., are those containing eighteen carbon atoms in the chain, while the sugar most commonly present is a hexose. It seems likely that the higher fatty acids may be synthesized in more than one way and that the intermediate ones may be formed either by synthesis from the lower ones and the elementary substances or by degradation from the higher members.

Fat Excretion

Probably no one of the foodstuffs is completely burned in the animal organism. The occurrence in the urine of residues of the protein molecule which still have some caloric value—urea, uric acid, traces of amino acids, etc.—is well known. The much debated question of the presence of sugar in normal urine has recently been convincingly answered in the affirmative by Benedict. Traces of fatty acids are present in normal urine but except in rare conditions the amounts found are not important. Fatty material, mainly in the form of fatty acids, is always present in the feces in considerable amounts. This fat may come from at least three sources: (a) undigested material from the food, (b) from the cellular material of the gastro-intestinal tract—epithelial cells, bodies of bacteria, etc., and (c) a true excretion of unused or unusable fat. To what extent food fat passes the tract unabsorbed under normal conditions cannot be stated, but it seems likely from considerations discussed earlier in the chapter that fats suitable as regards consistency and composition are completely digested and absorbed. Some of the feces fat undoubtedly arises from cellular material, but there is also considerable evidence to show that there is a true excretion of fat into the intestine. In fasting, fat is present in the feces to the extent of about $\frac{1}{3}$ of the total dry matter. Isolated rings of intestine with their blood supply intact fill up with material similar to feces containing about 35 per cent of their content of fat, an amount, when calculated for the whole intestine, agreeing with the figure for fat in fasting feces (Hermann, 1889-90). Loops of intestine with one or both ends opening outside the abdominal wall secrete a fluid which contains fatty material. In some animals the excretion flows freely

and may be collected from the fistula; in others it is viscous and must be washed out. In one dog used by the writer in which the fistula (about 14 inches of jejunum) had been established for about a year, a total of 0.72 gm. of fatty material, mainly soaps, was collected from the fistula in five days. At least two kinds of soap were present, one in the form of soft lumps, being probably palmitate, and the other in solution yielding a liquid fatty acid and being probably oleate. Experimenters from time to time have reported cases in which more fat appeared in the feces than was present in the food.

The Carbohydrates and Their Metabolism . . . . . .

. . . . . *A. I. Ringer and Emil J. Baumann*

Introduction—Chemistry of the Carbohydrates—Classification and Nomenclature—Constitution—Isomerism and Asymmetry—Mutarotation—Isomerism of the Aldohexoses—Chemical Reactions of the Carbohydrates—Synthesis and Degradation of Carbohydrates—Glucosides—Special Properties of Monosaccharides—Hexoses—Methyl Glucosides—Pentoses—Disaccharides—Polysaccharides—Digestion of Carbohydrates—Salivary Digestion—Action of Ptyalin—Gastric Digestion of Carbohydrates—Intestinal Digestion of Carbohydrates—Absorption of Carbohydrates—The Sugar of the Blood—Carbohydrate Tolerance—Carbohydrate Tolerance Standard—Glycogenesis and Carbohydrate Tolerance—Glucolysis and Carbohydrate Tolerance—Endocrine and Nerve Control of Glycogenesis, Glycogenolysis and Glucolysis—Influence of the Thyroid Glands—Influence of the Pituitary Gland—The Intermediary Metabolism of Carbohydrates—The Formation from Carbohydrate—The Function of Carbohydrate in the Diet—Influence of Carbohydrate on Intermediary Metabolism of Fat—Antiketogenesis.

The Carbohydrates and Their Metabolism

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1. Introduction

The carbohydrates, or sugars as they are called, are found in all cells. The name sugar is commonly applied to anything having a sweet taste, as sugar of lead for lead acetate. It is now used non-technically for some of the simpler members of this group—milk sugar (lactose), cane sugar (sucrose), etc. The generic name carbohydrate is derived from the fact that these substances are composed of the elements carbon, hydrogen and oxygen, the latter two being in the proportion in which they exist in water—two atoms of hydrogen to one of oxygen—in most, though not all cases; in other words, they are hydrates of carbon or *carbohydrates.*

In the plant world the carbohydrates are found serving two main functions: first, they act as the main constituent of supporting tissues or framework of the cell—cellulose; second, reserve food is stored up in this form as starches. In the animal world, carbohydrates no longer act as supporting structures of cells. Nitrogenous substances, belonging mainly to the class called proteins, take the place of them, but they are found as a form of reserve food—glycogen or animal starch. It is interesting to note that in some of the lower animal forms (in some molluscs), the supporting tissue, chitin, is a substance that may be considered as an intermediate of the proteins and carbohydrates. It is a nitrogenous carbohydrate from which glucosamine can readily be obtained. Carbohydrates are also found in the nuclei of all cells, in nucleic acids, and one of the simplest sugars, glucose, is almost always present in tissue fluids. They are the simplest organic substances found in living matter and the most abundant. All the more complex constituents of cells are derived from them ultimately.

2. Chemistry of the Carbohydrates

Classification and Nomenclature.—The carbohydrates, as has already been indicated, are composed of carbon, hydrogen and oxygen, usually having the formula $C_xH_{2n}O_n$. There are many substances having this generic formula that are not carbohydrates, e. g., CH_3-CHOH-COOH (lactic acid), but a more comprehensive definition will develop as the subject is presented.

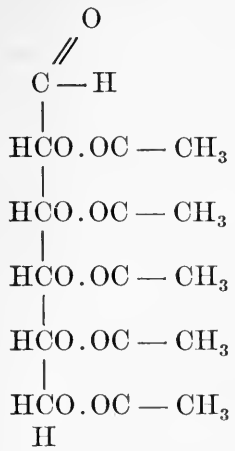
Carbohydrates may be divided into three great groups, according to the number of saccharide groups (simple sugars) they contain: monosaccharides, disaccharides, polysaccharides. Important monosaccharides are d-glucose or grape sugar, d-fructose or levulose, d-mannose, d-arabinose and d-ribose. Common disaccharides are sucrose or cane sugar (also known as saccharose), lactose or milk sugar, and maltose or malt sugar. These comparatively simple carbohydrates are often called sugars. Common polysaccharides are cellulose, starches, dextrins, glycogen and gums.

The monosaccharides are further divided according to the number of carbon atoms they contain—trioses, pentoses, hexoses, octoses, nonoses, etc. Those found occurring in nature are chiefly the tetroses, pentoses, hexoses and a few heptoses. Some of the carbohydrates have the properties of an alcohol and aldehyde, others of an alcohol and a ketone, and these are known respectively as aldoses and ketoses. So an aldehyde sugar having six carbon atoms would be called an aldo-hexose, and a ketone sugar having six carbon atoms would be called a keto-hexose.

Constitution.—In the discussion of the structure of the carbohydrates, d-glucose will be used as a typical example of the aldoses. The manner in which the elements carbon, hydrogen and oxygen are combined in these compounds has been a problem which has gradually been elucidated during the last century, although the last word on the subject has not yet been written. The first step in the solution of the problem may be said to have been devised by Liebig, when he gave forth his method for determining the percentages of carbon and hydrogen in organic matter. With the development of definite concepts of valency by Kekulé and others, and of the asymmetric carbon atom by Le Bel and Van't Hoff in 1875, a fairly definite idea of the structure of these substances became known.

As shown by elementary analysis, glucose has the empirical formula CH_2O, and the molecular formula, $C_6H_{12}O_6$, as shown by molecular weight determinations, by the cryoscopic and ebullioscopic methods. When treated with acids, acid anhydrids and acid chlorides, glucose forms ethereal salts or esters,[1] e.g., acetyl chloride will form a glucose pentacetate, $C_6H_7O(O.CO.CH_3)_5$.

[1] Alcohols are compounds of carbon containing one or more hydroxyl groups, as CH_3OH, methyl alcohol. An organic acid is a compound containing a carboxyl group



That is, glucose behaves like a compound having five alcohol (OH) groups here, and Berthelot, who first prepared the acetates of glucose, called the sugar a pentatomic aldehyde alcohol. When acted upon by metallic hydroxides, glucose forms compounds resembling alcoholates, further demonstrating the presence of alcohol groupings.

Glucose is reduced by sodium amalgam to form a hexahydric alcohol, which in turn may be reduced by hydriodic acid to iodohexane, a derivative of normal hexane, which indicates that glucose is a normal chain com-

(COOH). Acids and alcohols react forming ethereal salts or esters, much as acids and bases react to form salts, thus:

$$\underset{\text{methyl alcohol}}{CH_3OH} + \underset{\text{acetic acid}}{CH_3COOH} \rightleftarrows \underset{\text{methyl acetate (ester)}}{CH_3COOCH_3}$$

Substances having the group $-C\begin{matrix}O\\ \diagup\!\!\diagup\\ H\end{matrix}$ are called aldehydes, and those that contain the carbonyl group $\begin{matrix}|\\ CO\\ |\end{matrix}$ are known as ketones. A fundamental distinction between aldehydes and ketones, is that when they are oxydized, aldehydes yield acids containing the same number of carbon atoms as the original substance while ketones break up on oxidation, yielding products which do not contain as many carbon atoms as the original substances. Thus:

$$CH_3CH_2C\begin{matrix}O\\ \diagup\!\!\diagup\\ H\end{matrix} + O \rightarrow CH_3CH_2COOH$$

propyl aldehyde — propionic acid

$$\begin{matrix}CH_3\\ \diagdown\\ \quad CO\\ \diagup\\ CH_3\end{matrix} + 3\,O \rightarrow CH_3COOH + HCOOH$$

methyl ketone (acetone) — acetic and formic acids

TABLE I.—CLASSIFICATION OF CARBOHYDRATES

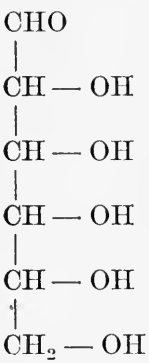
- **Carbohydrates**
  - *1. Monosaccharides*
    - 1. Bioses — aldose (glycolicaldehyde)
    - 2. Trioses
      - aldose (glycerose)
      - ketose (dioxyacetone)
    - 3. Tetroses
      - aldose (erythrose)
      - ketose (erythrulose)
    - 4. Pentoses
      - aldoses (arabinose, xylose, ribose)
      - ketose (arabinulose)
    - 5. Hexoses
      - aldoses (glucose, galactose, mannose)
      - ketoses (fructose, sorbose)
    - 6. Heptoses
      - aldoses (mannoheptose, glucoheptose)
  - *2. Disaccharides*
    - Type 1. *Aldehyde group functional*
      - Maltose (glucose and glucose)
      - Isomaltose (glucose and glucose)
      - Lactose (glucose and galactose)
      - Turanose (glucose and fructose)
    - Type 2. *Aldehyde not functional*
      - Sucrose (glucose and fructose)
      - Trehalose (glucose and glucose)
  - *3. Polysaccharides*
    - 1. Trisaccharides
      - Type 1. Mannotriose (glucose and galactose and galactos
      - Type 2.
        - Raffinose (galactose and glucose and fructose)
        - Melicitose (glucose and glucose and fructose)
    - 2. Tetrasaccharides
      - Stachyose (fructose and glucose and galactose and galactose)
    - 3. Colloidal Polysaccharides
      - Dextrins
      - Glycogen
      - Starches
      - Celluloses
      - Gums

pound. By oxidizing glucose with bromine, gluconic acid is obtained. This has the same number of carbon atoms as glucose, and in this way the presence of an aldehyde is indicated, a fact which is confirmed by oxidizing glucose with nitric acid to saccharic acid, a dicarboxylic acid, also containing six carbon atoms.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + O \longrightarrow \underset{\text{Gluconic Acid}}{C_6H_{12}O_7}$$

$$\underset{\text{Gluconic Acid}}{C_6H_{12}O_7} + O \longrightarrow \underset{\text{Saccharic Acid}}{C_6H_{10}O_8}$$

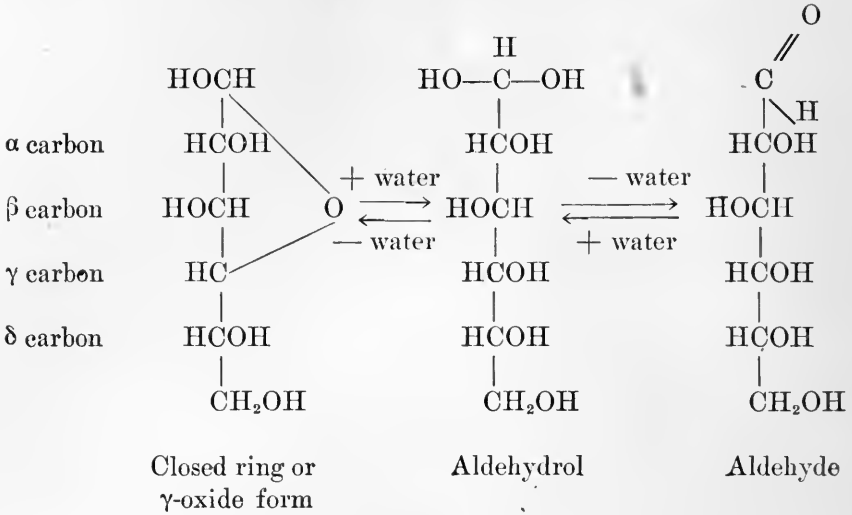
Owing to the stability of glucose it may be assumed that each hydroxyl group is attached to a different carbon atom, and as glucose is a derivative of normal hexane, as shown above, its formula may be written



This formula was originally proposed by Baeyer (1) and Fittig (2)[2] But glucose is far less active than might be expected of a compound that is an hydroxyaldehyde. Thus it does not react easily with sodium sulphite, pyrotartaric acid, nor with phenylhydrazineparasulphonic acid as might be expected of a substance having the formula shown. It does not undergo Perkins' reaction for aldehydes with acetic anhydride and sodium acetate. Aldehydes are generally more volatile than the corresponding alcohols. This is not true of glucose. Moreover, glucose and many of its derivatives, as shall be seen presently, occur in two isomeric forms which exhibit no aldehyde properties at all. This difficulty was overcome by Tollens' (1883) suggestion of a ring (the γ-oxide or γ-lactone) formula for glucose. This formula has now been generally adopted. On

[2] The presence of a ketone group (CO) in carbohydrates was first demonstrated by Kiliani in 1885 when he showed that, unlike glucose, which owing to its aldehydic nature yields compounds with the same number of carbon atoms when oxidized, fructose, under similar conditions, yeilds a number of products having less than the same number of carbon atoms than the original substance, as, for instance, trihydroxybutyric acid.

the basis of this configuration it is assumed that glucose may readily behave like an aldehyde by breaking the γ-oxide ring, thus:

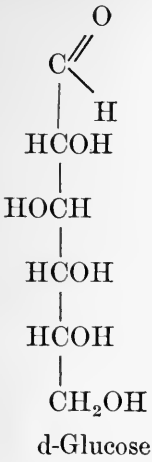


An intermediate aldehyde-hydrate or aldehydrol form is believed to result by hydrolysis, and from this in turn the aldehyde form originates. The action is a reversible one, and it is assumed that when an agent that will act upon the aldehyde group is added to an aqueous solution of glucose, the small amount of aldehyde-hydrate present is acted upon, thereby disturbing the equilibrium. A fresh quantity of the hydrate is formed and so the process is kept up.

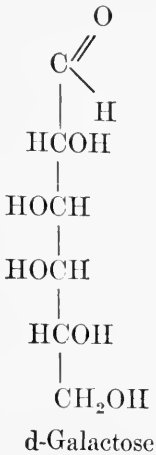
Isomerism and Asymmetry.—Bodies having the same elementary composition, but possessing different properties, are called isomers or isomerides. Thus ethyl alcohol CH_3 | CH_2OH and methyl ether CH_3 \ O / CH_3 are isomers. Both have the empirical formula of C_2H_6O. When, however, in addition to having the same number of atoms of the same kind, these atoms are arranged in the same general way, so that each compound has the same chemical groups, and consequently similar chemical properties, but the "space relationships" of these groups within the molecule are different, such substances are said to be *stereoisomeric.*

Sugars illustrate this form of isomerism especially well. For example, glucose and galactose are both aldohexoses. They have the same empirical formulæ and the same chemical groups, but the space relationships or configuration of these groups differ.

These differences are illustrated in the following structural formulas:



d-Glucose



d-Galactose

Pasteur was the first to clearly demonstrate the importance of the relationship of the atoms to one another in the molecule, and added one of the most fundamental facts concerning the structure of the molecule to the chapter of chemistry. To biochemistry, or for that matter to all medical sciences, Pasteur's contribution on this fascinating subject is of supreme importance, and to-day we are really only beginning to appreciate how important molecular structure is in metabolism.

While Pasteur was studying crystalline structure (in 1848) he investigated the tartaric acids. Two forms of tartaric acid were known then—that obtained from wine, which rotated the plane of polarized light to the right, and that, called racemic acid, having the same composition, and no action on polarized light. He expected that these two forms of tartaric acid would have different crystalline forms. He worked with the sodium ammonium salts of these acids and found that the ordinary tartaric acid from grapes had pretty much the same form as racemic acid. However, closer examination of the crystals of racemic acid showed that there were really two types present, one having a pair of diagonally opposite facets so arranged that if superimposed upon the other, these facets would not correspond. In the one type, one of these facets was on the right side, and in the other type of crystal, the corresponding facet was on the left side. And one of the forms of racemic acid proved to be the same as the optically active tartaric acid obtained from wine.

Pasteur then separated the two types of crystals found in racemic acid, studied their behavior toward polarized light, and discovered that in one case the plane of polarized light was rotated to the right, and in the other the plane of polarized light was rotated to the left. The difference between the two forms of tartaric acid thus became apparent. The natural tartaric acid rotates the plane of polarized light to the right;

the form isolated by Pasteur from racemic acid rotates the plane of polarized light to the left; racemic acid, optically inactive, is in reality a mixture of both—the dextrorotatory and the levorotatory.

Here are two substances having the same empirical formula and the same chemical groups similarly arranged, but their physical properties—their crystalline form and behavior toward polarized light—are markedly different. It will likewise be found that their chemical properties are different. These are not due to differences in chemical composition, but to differences in molecular form. More than a quarter of a century later, Le Bel and Van't Hoff independently formulated the hypothesis of the asymmetric carbon atom, on the basis of Pasteur's fundamental discovery. Only such compounds of carbon as have so-called asymmetric

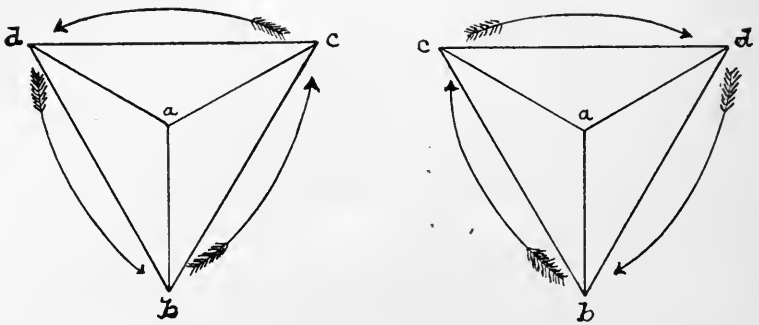
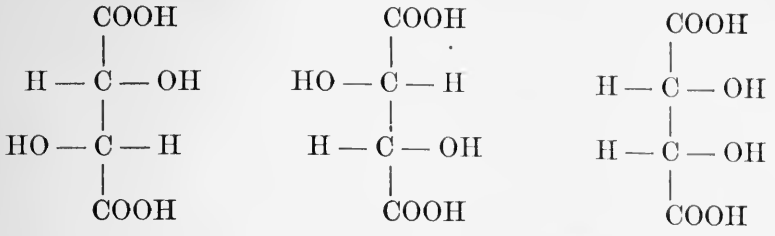


Fig. 1. Illustrating two carbon atoms with their four valences taken up by four different radicles arranged in such a way that the space relationship of the two is like that of a mirror image.

carbon atoms can exist in stereoisomeric forms. An asymmetric carbon atom is one that has four different atoms or atomic groups attached to it.

If the carbon atom is pictured as lying at the center of a tetrahedron with the four atoms attached to it at the apices, it is possible to arrange these in two ways, one of which is the mirror image or antipode of the other (Fig. 1).

Molecular asymmetry of this type is most readily recognized by means of the action of such substances on polarized light. Compounds having one or more asymmetric carbon atoms usually have the power of rotating the plane of polarized light except when one asymmetric carbon atom is neutralized by one or more other asymmetric atoms. However, one does not meet such substances very often. One of the first cases known in which one asymmetric carbon atom neutralizes another is mesotartaric acid, discovered by Pasteur. The various tartaric acids may be represented thus:



d-Tartaric acid | l-Tartaric acid | Mesotartaric acid

It is found that optical antipodes rotate the plane of polarized light in equal amounts but in opposite directions, so that, if one has a mixture of equal parts of the dextro- and levorotatory forms of a compound, the resulting mixture would of course exert no influence upon the plane of polarized light.

The degree of rotation varies directly as the concentration of the substance and inversely as the length of the column of solution through which the observation is made. It depends also upon the temperature (there being less rotation in general as the temperature increases) and on the wave length of the light used in making observations. The degree of rotation for many substances is greater with light of short than of long wave lengths. Hence the necessity of using a standard temperature and a monochromatic source of light for making observations. The unit of measurement of rotation of the plane of polarized light is called the specific rotatory power and is defined as the rotation of one gram of substance dissolved in one cubic centimeter of solute and for a tube one decimeter in length, usually at 20 degrees centigrade and for sodium light. It is calculated from the observed angle of rotation, produced by a solution of known concentration, in a tube of known length, by the following formula:

$$[\alpha]_{D}^{20} = \frac{\alpha}{P \cdot l}$$

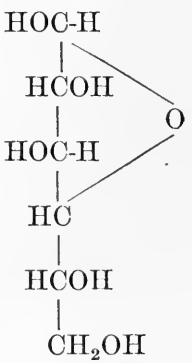
in which $[\alpha]_{D}^{20}$ is the symbol for specific rotation at 20° for sodium light (the D line of the spectrum), α the observed angle of rotation, P the concentration of the substance, and l the length of the tube in decimeters. The solvent is usually given, as the angle of rotation varies somewhat with different solvents.

Mutarotation.—*Isomerism of Glucose.*—When pure d-glucose, derived from natural sources, is dissolved in water, and its specific rotation determined at once, it will be found to be +109°. On standing, the specific rotatory power changes slowly, until after 24 hours or more, at 20°, it becomes +52.5°. If a small quantity of alkali is added to the newly

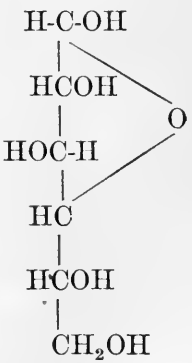
prepared solution, this change will take place in a few minutes. This phenomenon was first observed by Dubrunfaut in 1846. By crystallizing ordinary commercial glucose from different solvents and by other methods, two different glucoses have been obtained, having specific rotatory powers of + 109 and + 19 respectively. If either of these is dissolved in water, it will slowly change its specific rotation to + 52.5. This phenomenon is termed mutarotation or birotation.

Tanret, in 1895 and 1896, was the first to demonstrate that we were here dealing with more than one form of glucose. He called the glucose with the high initial specific rotation α glucose, and the glucose of the specific rotatory power 52.5 he designated β-glucose. However it has been found that Tanret's β-glucose was really a mixture obtained by allowing the glucose of high or low rotatory power to reach equilibrium. This happens when there are present 37 per cent of α-glucose and 63 per cent of the glucose having the initial specific rotatory power of +19, which is now called β-glucose. The equilibrated mixture of α-and β-glucose is known as γ-glucose.

The difference in structure of α and β-glucose is due to the difference in the positions of the hydrogen atom and hydroxyl group of the carbon atom that is potentially aldehydic. It may be represented as follows:



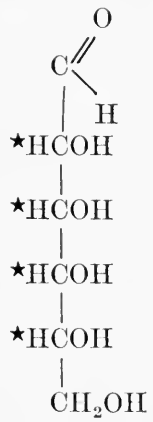
α-Glucose



β-Glucose

The conversion of one form to the other is assumed to take place by the formation of an intermediary compound, the exact nature of which is still a matter of dispute.

Isomerism of the Aldohexoses.—The number of possible stereoisomeric forms of a substance can be calculated by the formula of Le Bel and Van't Hoff. Number $= 2^n$, where n is the number of asymmetric carbon atoms in the molecule. If the open chain formula of glucose is examined it will be found that it has four asymmetric carbon atoms:



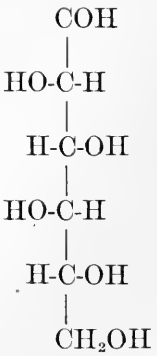
Accordingly there may be 2^4 or 16 possible aldohexoses. Largely through the researches of Emil Fischer, 14 of these are now known, although only three—glucose, mannose, galactose—occur naturally. These isomers are represented in Table II.

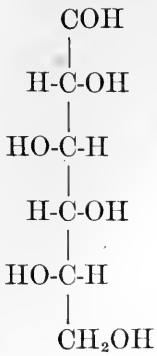
TABLE II.—ALDOHEXOSES

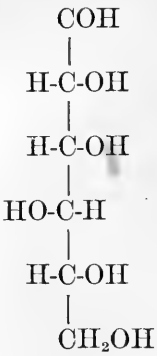
1. Mannitol Series

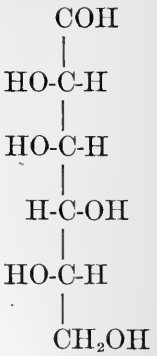
l-Mannose	d-Mannose	l-Glucose	d-Glucose [3]
COH	COH	COH	COH
H-C-OH	HO-C-H	HO-C-H	H-C-OH
H-C-OH	HO-C-H	H-C-OH	HO-C-H
HO-C-H	H-C-OH	HO-C-H	H-C-OH
HO-C-H	H-C-OH	HO-C-H	H-C-OH
CH_2OH	CH_2OH	CH_2OH	CH_2OH

[3] All sugars known as d-sugars are not necessarily dextrorotatory, nor are all l-sugars necessarily levorotatory. All compounds derived from d-glucose by reactions that leave the stereochemical structure unchanged are designated d-compounds, regardless of their rotation, and similarly for l-forms.

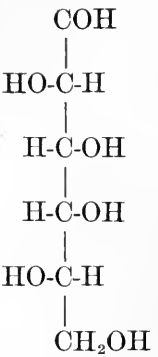

l-Idose

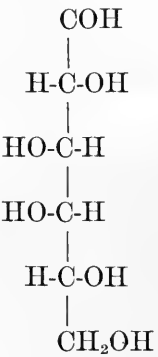

d-Idose


l-Glucose


d-Glucose

2. Dulcitol Series


l-Galactose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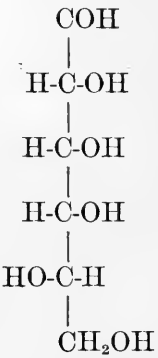
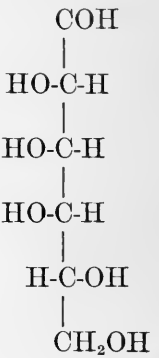

l-Talose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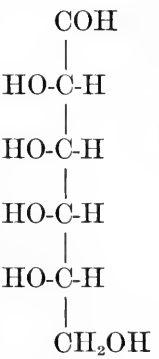
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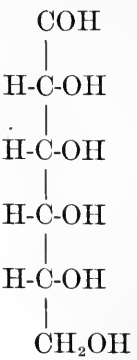
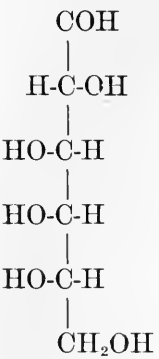
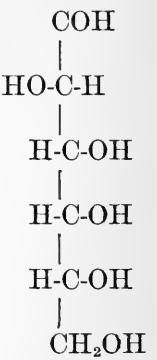

d-Allose


l-Altrose
unknown


d-Altrose

Since there are two closed ring forms for each aldohexose, the α and β forms, there should be 32 closed chain aldehexoses,[4] with which the 16 already discussed make a total of 48 isomeric aldohexoses theoretically possible. Most of the carbohydrates exist in more than one form and possess the power of mutarotation.

TABLE III

Specific Rotations of Sugars

Sugars	α-form	β-form	Equilibrated Mixture
d-Glucose	+ 110°	+ 20°	+ 52.5°
d-Mannose	+ 76°	— 14°	+ 14°
d-Galactose	+ 140°	+ 53°	+ 81°
d-Fructose	+ 17°	— 140°	— 93°
l-Arabinose	+ 76°	+ 184°	+ 104°
d-Xylose	+ 100°	— 8°	+ 19°
l-Rhamnose	— 7°	+ 32°	+ 9°
d-Maltose	+ 166°	+ 119°	+ 137°
d-Lactose hydrate	+ 86°	+ 35°	+ 55°
d-Melibiose	+ 171°	+ 124°	+ 143°
d-Ribose	.....	.....	+ 18.8°
Sucrose	.....	.....	+ 66.5°

α Methyl glucoside + 157°. β Methyl glucoside — 33°.

Chemical Reactions of the Carbohydrates

In most cases glucose will be used as a typical carbohydrate in discussing the reactions which the carbohydrates undergo. (Only those that have a direct interest to the biochemist will be presented.)

Synthesis and Degradation of Carbohydrates.—Most of the methods of synthesizing the carbohydrates we owe to the masterly researches of Emil Fischer, who devised most of the methods and synthesized a vast number of them.

1. *Polymerization (aldol condensation) of simple sugars by action of dilute alkali,* e.g.,

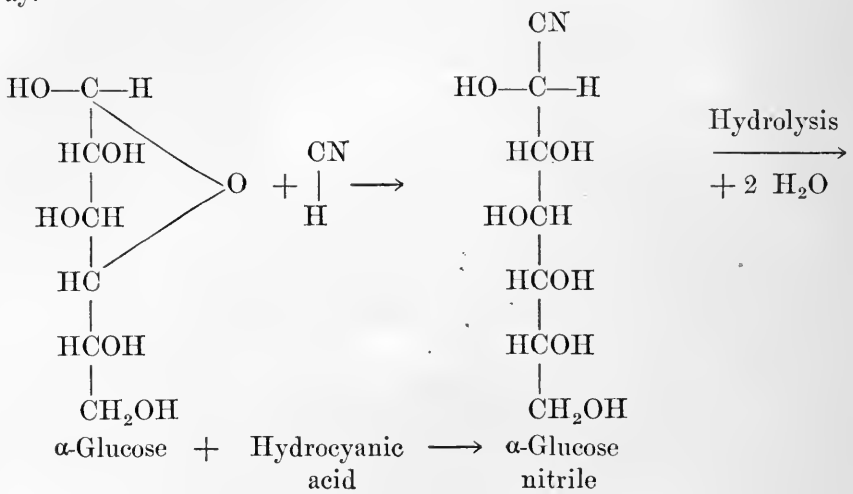
$$\underset{\text{Glycerose}}{2\,C_3H_6O_3} \rightarrow \underset{\text{Fructose}}{C_6H_{12}O_6}$$

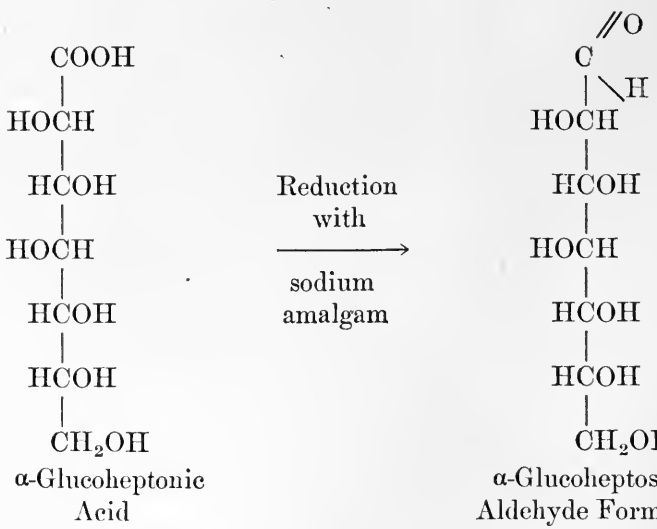
This reaction is somewhat similar to one by which it is believed carbohydrates may be formed in plants from formaldehyde. Baeyer, in 1870, first advanced the theory that the plant tissues formed formaldehyde from CO_2 and H_2O. Loew, in 1886, discovered that formaldehyde (HCOH) and lime water at room temperature produced a sweet substance which was unfermentable. Fischer later showed that what is formed here is α acrose,

[4] In the closed chain formula there is an additional asymmetric carbon atom, so that the number of isomers is 2^5 or 32.

which is the inactive form of fructose, so that chemically at least this is a possible mechanism by which plants synthesize carbohydrates.

2. *Synthesis of higher forms from a lower monosaccharose.*—Here, a method of wide application in chemistry has been successfully used to synthesize a large number of carbohydrates. It consists in forming a cyanhydrin of a lower aldose with hydrocyanic acid, hydrolyzing the nitrile to form the corresponding acid and reducing this substance to the next higher sugar, e. g., glucose may be converted to glucoheptose in this way.

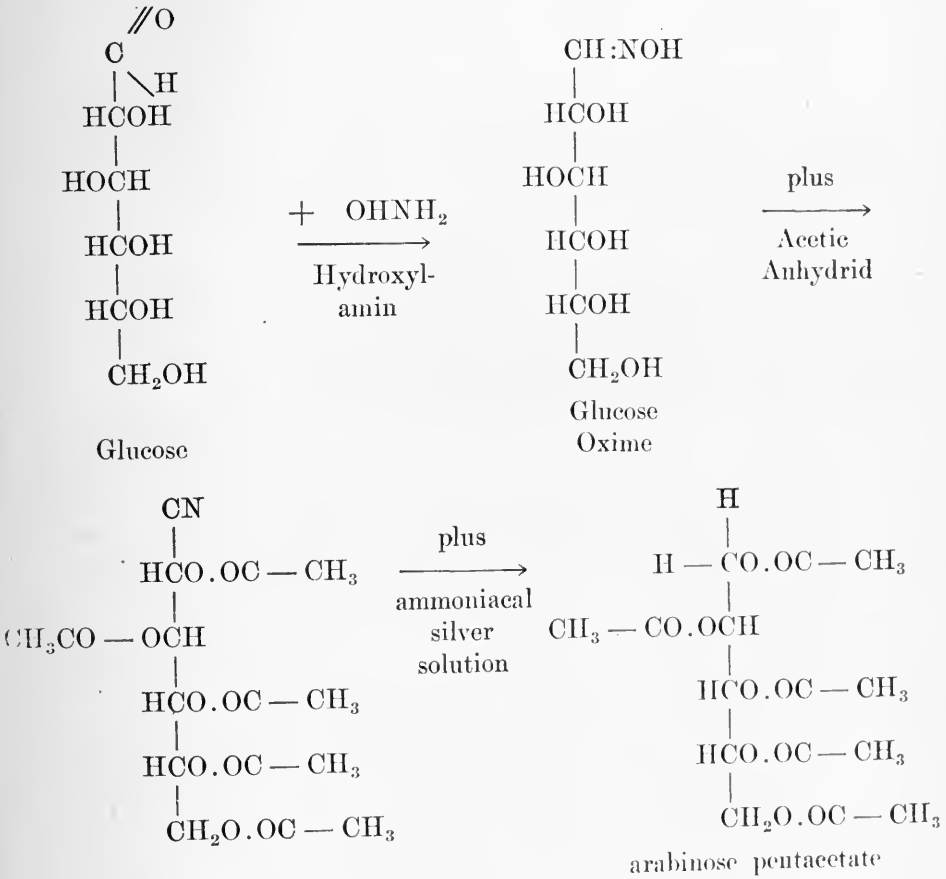




The ability of hydrocyanic acid to unite with aldoses is of considerable interest physiologically. This acid is found in small amounts in a number

of plant tissues. It greatly accelerates the action of a proteolytic enzyme (papain) which it may do by means of a reaction somewhat similar to the first stage indicated above.

3. *Conversion of a higher to a lower monosaccharose.*—By the action of hydroxylamine upon glucose, glucose-oxime is produced. This product is converted to gluconic nitrile by the action of acetic anhydrid and sodium acetate, removing one molecule of water and acetylating the hydroxyl groups, forming penta-acetyl gluconic acid. Ammoniacal silver solution removes hydrocyanic acid from this substance, leaving the acetyl derivative of the pentose arabinose. Ammonia will form an acetamid arabinose, which in turn yields arabinose by the action of dilute sulphuric acid.



By this reaction glucose has been converted successively into arabinose, erythrose, glycerose and glycollic aldehyde.

Oxidation. Action of alkalies.—Most of the simpler carbohydrates are unstable in alkaline solution and undergo a great variety of changes.

the exact nature of them all not being known yet. If the sugars are treated with a weak alkali at room temperature, a molecular rearrangement takes place slowly which is known as a tautomeric rearrangement. The mechanism of these interesting changes will be presented later. If an aldose or ketose is treated with strong alkali, it becomes yellow or brownish and acquires the odor of caramel. This is the basis of Moore's test for the detection of carbohydrates. The character of the products formed varies with the strength of alkali used and the amount of oxygen available, for the products are largely oxidation products, the sugar being a reducing agent. Over one hundred degradation substances have been identified as the products of the interaction of sodium hydroxid and glucose.

Among others, a large series of acids may be formed, varying in complexity from carbonic acid, formic acid, oxalic and lactic acids, to saccharic and gluconic acids. In the absence of much oxygen, products like glycolic-aldehyde CH_2OH, CHO, glycericaldehyde CH_2OH, $CHOH$, CHO, glyoxal CHO, CHO, oxyacetone CH_2OH, $C{=}O$, CH_3, etc., are formed.

The first stages in the oxidation of glucose results in the formation of gluconic and glucuronic acids—both monocarboxylic acids, and then saccharic acid—a dicarboxylic acid.

Glucose	Glucuronic Acid	Gluconic Acid	Saccharic Acid
CHO	CHO	COOH	COOH
CHOH	CHOH	CHOH	CHOH
CHOH	CHOH	CHOH	CHOH
CHOH	CHOH	CHOH	CHOH
CHOH	CHOH	CHOH	CHOH
CH_2OH	COOH	CH_2OH	COOH

(Glucuronic acid is the most interesting of these derivatives physiologically. Many substances that are not readily oxidized in the body, such as camphor,

chloral, thymol or phenol, are excreted in the urine of the carnivora and herbivora as conjugated glucuronates. These glucoside [5] compounds serve as a means of removing injurious substances from the body. In the plant kingdom, glucuronates have also been found frequently, e. g., in the sugar beet.)

As one would expect of ketones, the ketohexoses do not yield acids containing the same number of carbon atoms on oxidation. The molecule divides at the ketone group.

Monosaccharides, and many disaccharides and trisaccharides, are oxidized in acid solution, forming products similar to those formed by the action of alkali, but the oxidation occurs much less readily.

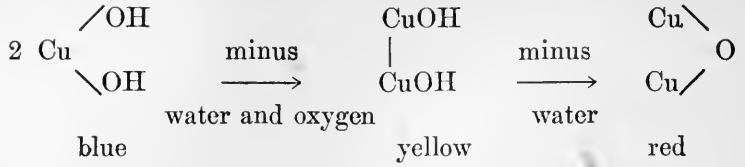
These reducing powers of the simpler carbohydrates are utilized in detecting and estimating them quantitatively. In alkaline solution they will reduce many metallic hydroxides, such as those of copper, mercury, bismuth, silver, gold, etc. Methylene blue, permanganates, bromin, chlorin, etc., are also reduced by sugars, the last three in acid solution as well as in alkaline solution.

The carbohydrates are usually estimated quantitatively or detected qualitatively by an alkaline cupric tartrate solution, known as Fehling solution or some modification of it. If glucose be heated with cupric hydroxid [$Cu(OH)_2$] and sodium hydroxid, it will reduce some cupric hydroxid to cuprous oxid [Cu_2O]. When much cupric hydroxid is present it will remain partly dissolved and some of it may be dehydrated to form black cupric oxid [CuO].

Many substances, usually those having several hydroxyl groups, such as tartrates, citrates, glycerol and sugars, possess the property of dissolving metallic hydroxids, as in the case of sodium tartrate and $Cu(OH)_2$ forming cupric tartrate. If enough sodium tartrate be added to cupric hydroxid and sodium hydroxid, all the cupric hydroxid will dissolve. When glucose is heated with such a solution reduction of the cupric hydroxid will occur with no danger of formation of cupric oxid, which might obscure the result. Fehling's solution is an alkaline cupric tartrate solution made from copper sulphate, sodium potassium tartrate (Rochelle salt) and sodium or potassium hydroxid. When kept for any length of time, the tartrate will reduce the cupric salt. To avoid this the copper sulphate is kept separate and is known as Fehling's solution "A" and the alkaline tartrate solution as Fehling's solution "B".

The stages in the reduction of copper by reducing sugars are roughly as follows: the alkali decomposes the sugar into a number of fragments which reduce the cupric salt to insoluble yellow cuprous hydroxid, first. If heating is continued, the cuprous hydroxid loses a molecule of water and is converted into red cuprous oxid, which is also insoluble.

[5] A glucoside is an ether of glucose (or other sugars) and an alcohol. On hydrolysis with acid, the sugar is liberated.



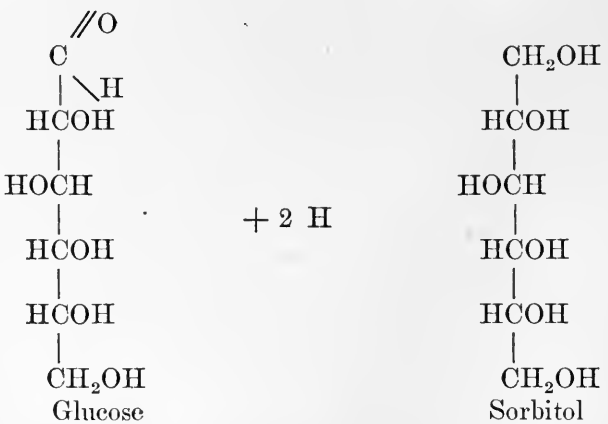
It should be noted that in Fehling's solution both cupric hydroxid and cupric tartrate exist in equilibrium. As reduction occurs, more cupric hydroxid is formed from the tartrate.

This reaction is not completed in a definite time, since many of the degradation products, as gluconic acid, are slowly oxidized. So that when quantitative estimations are made, very definite conditions of concentration and time of heating must be observed. The cuprous oxid formed may be weighed directly or oxidized to cupric oxid and this weighed. Or it may be dissolved in acid and estimated electrolytically or by a number of volumetric methods.

To avoid the inconvenience of keeping two solutions, Benedict has substituted sodium citrate for Rochelle salts in Fehling's solution and sodium carbonate for sodium hydroxid. This solution keeps indefinitely and serves very well for the qualitative detection of reducing substances.

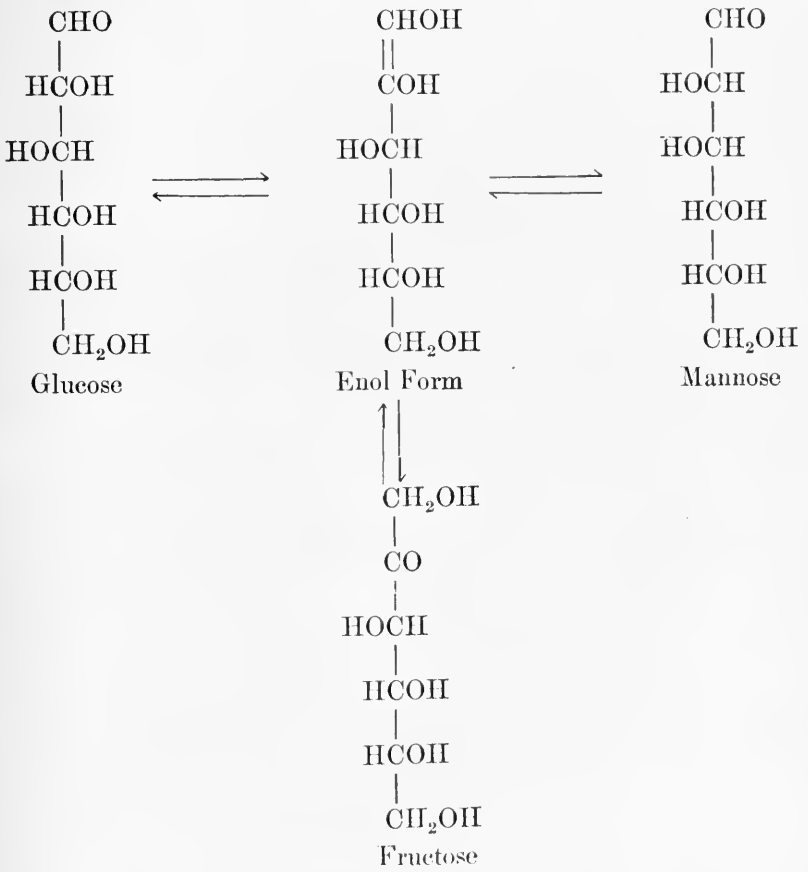
Reduction of Carbohydrates.—While most of the reactions which carbohydrates undergo in living matter are oxidation reactions, not an inconsiderable number are reductions, such as the processes whereby microorganisms, of the group known as anaërobes, metabolize sugars and give off carbon dioxid in the absence of air.

Sugars are reduced by sodium amalgam, forming, in the case of hexoses, hexahydric alcohols.

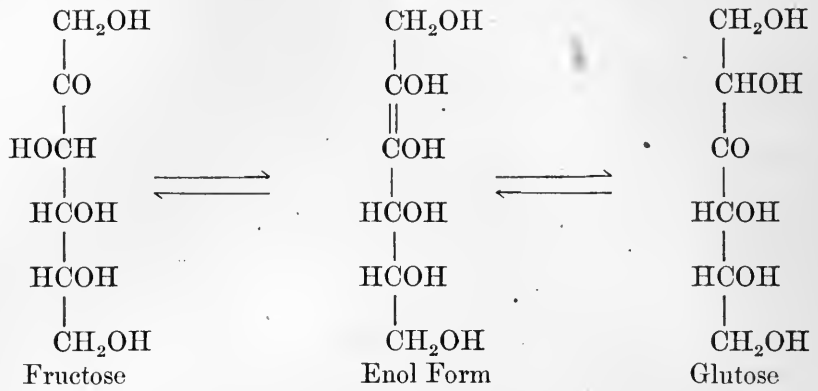


A number of these alcohols are found in plants, such as sorbitol, which is derived from glucose; mannitol from mannose; dulcitol from galactose. Mannitol is especially widely distributed. In some fungi there is more mannitol present than glucose. Like the sugars, they are sweet.

Conversion of Glucose into Fructose and Mannose.—In the presence of alkalis, aqueous solutions of glucose, mannose and fructose are converted into one another; slowly at room temperature, more quickly and with some decomposition at higher temperatures. These most interesting and important reactions were first observed by Lobry de Bruyn and A. Van Ekenstein, 1902-1903. They noticed that if glucose were treated with weak alkali at room temperature, the specific rotation changed from + 52.5° to about 0°. After standing several days or weeks, mannose and fructose, as well as glucose, could be isolated from the solution. The mechanism of the process was explained by Wohl. It will be remembered that except for the terminal and α-carbon atoms, the space configuration of glucose, fructose and mannose is the same. The hydrogen atom attached to the α-carbon in glucose and mannose "swings" from its position to give rise to the common enol form. In the case of fructose the swinging H atom is attached to the terminal C atom. The enol form is then converted into all three of the possible hexoses.



Lobry de Bruyn isolated another hexose, glutose, as a product of the action of alkali on glucose. Glutose is formed through the intermediate stage of a second enolic form derived from fructose, thus:

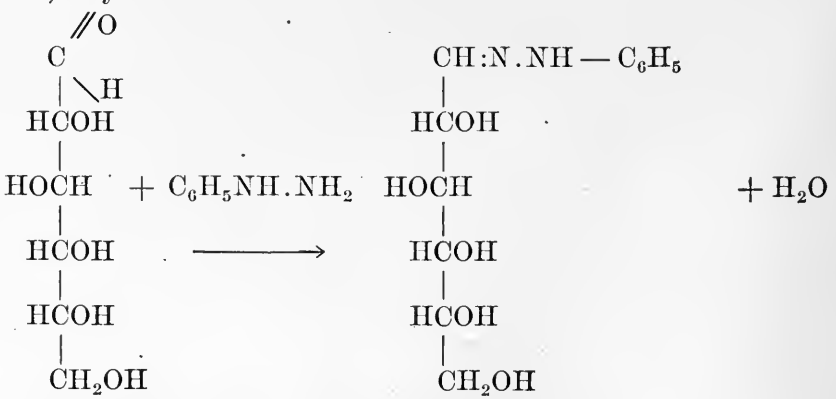


Fructose Enol Form Glutose

d-Galactose behaves similarly to d-glucose when treated with dilute alkalis. An equilibrium ensues between it and d-talose, d-tagatose and l-sorbose.

Reactions of sugars with Substituted Hydrazines.—One of the most important reactions in sugar chemistry for identification of sugars is that which takes place when aldoses or ketoses are heated with an excess of phenylhydrazine in dilute acetic acid. Quite insoluble definite crystalline compounds are formed, called hydrazones and osazones, which are readily identified by their crystalline structure, melting point, etc. These osazones (and hydrazones) were the compounds that enabled E. Fischer to elucidate the chemistry of the sugars.

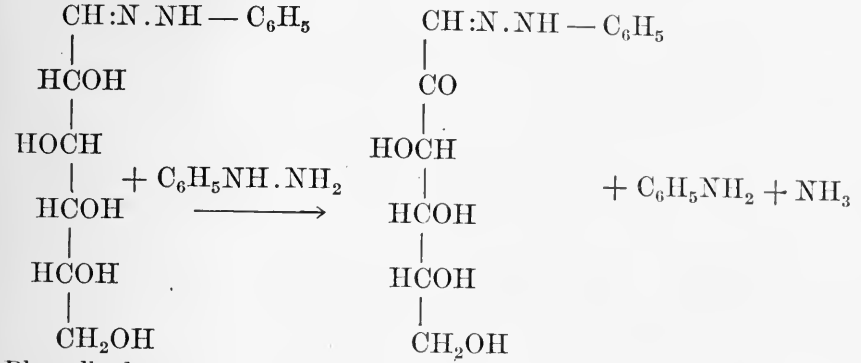
The reaction takes place in two stages. In the first, which goes on at 20° C., a hydrazone is formed.



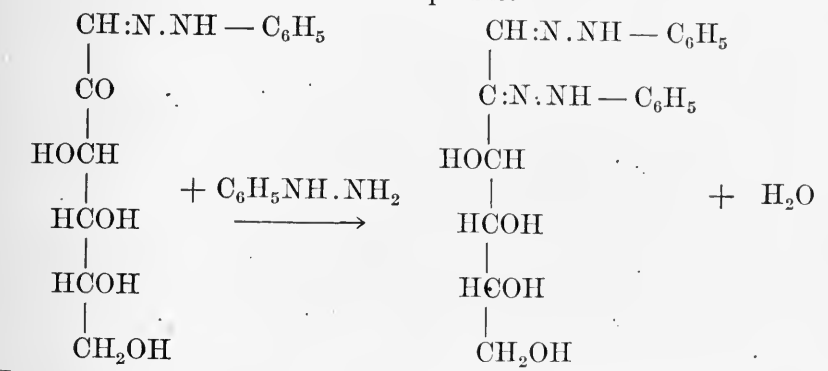
Aldose (Glucose) Phenylhydrazine Phenylhydrazone

An excess of phenylhydrazine (which should be present) then acts as an oxidizing agent, forming a carbonyl group (CO) from a CHOH group,

while the phenylhydrazine is converted to anilin and ammonia. The carbonyl group then reacts with another molecule of phenylhydrazine to form the osazone, thus:



Phenylhydrazone + phenylhydrazine — intermediary oxidation product + anilin + ammonia



Intermediary oxidation product + phenylhydrazine — phenylosazone + water

Because the second stage of the reaction is a process of oxidation, it follows that those sugars that are most easily oxidized (as d-fructose) most readily form osazones.

Aldoses and ketoses may be differentiated by means of their reaction with methyl phenylhydrazine. According to Newberg, ketoses form osazones, while aldoses reach only the hydrazone stage. The asymmetrically substituted hydrazines do not act as oxidizing agents. Since the conversion of hydrazone to osazone involves oxidation, the reason for this behavior is evident.

Most of the hydrazones are very soluble in water and therefore not adapted for identification. Mannose, however, is a notable exception. It forms a crystalline precipitate easily identifiable. The osazones are, as a rule, quite insoluble in water. In order to form more specific com-

pounds for identification, disubstituted hydrazines have been used with excellent results in many cases. Thus, galactose forms a very characteristic methyl phenylhydrazone with methylphenylhydrazine. Other characteristic sugar compounds with the hydrazines are the diphenylhydrazone of arabinose, benzoylphenylhydrazones, etc.

Glucose, fructose and mannose form the same phenylosazone—glucosazone—as would of course be expected from their configuration, as previously noted (see page 231).

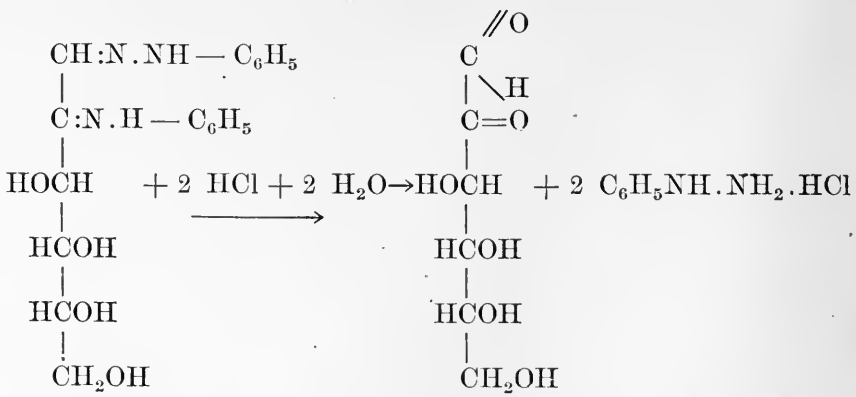
As stated above, the asymmetrically substituted hydrazines do not form osazones with glucose because they cannot act as oxidizing agents. Fructose, however, already having a CO group present, is readily attacked by them.

The osazones and hydrazones, then, form an admirable means of isolating carbohydrates from a solution containing inorganic and organic substances, i. e., biological fluids, like blood, urine, etc. To recover the free sugar from the hydrazone, Fischer decomposed them with hydrochloric acid into phenylhydrazine and sugar. It was later discovered that boiling them with benzaldehyde and water, in the case of the monosubstituted hydrazones, or with formaldehyde, in the case of the disubstituted hydrazones, was advantageous (Heizfeld, Ruff and Ollendorf), for then, insoluble benzaldehyde phenylhydrazone or formylphenylhydrazone were formed, and the phenylhydrazones could be removed by filtering off these insoluble derivatives.

$$C_6H_{12}O_5{:}N.NH{-}C_6H_5 + C_6H_5CHO \rightarrow C_6H_{12}O_6 + C_6H_5CH{:}N.NH{-}C_6H_5$$

Phenylhydrazone + benzaldehyde → sugar + benzaldehyde phenylhydrazone

Sugars cannot, however, be so readily recovered from their osazones. When the latter are treated with concentrated hydrochloric acid it will remove both hydrazine groups, forming an osone:



Phenylosazone + hydrochloric acid and water → osone + phenylhydrazine hydrochlorid

The osones are colorless liquids which act as strong reducing agents. By reducing them the sugars may be obtained. Glucose, fructose and mannose form the same osazone, and so, of course, the same osone. When glucosone is reduced, d-fructose is obtained. These reactions may therefore be used for converting an aldose into a ketose.

TABLE IV

MELTING POINTS

OF HYDRAZONES

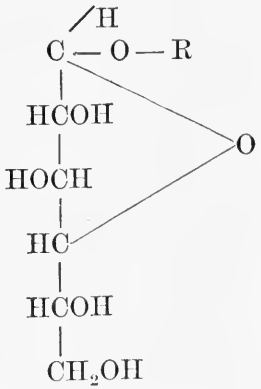
	Arabinose	Glucose	Mannose	Galactose	Maltose	Lactose
Phenylhydrazone	151-3°	144-6°	186-8°	158°	....	....
p-bromophenylhydrazone	150°	164-6°	208-10°	168°	....	....
α-methylphenylhydrazone	161°	130°	178°	180°	....	....
α-ethylphenylhydrazone	153°	....	159°	169°	....	....
α-amylphenylhydrazone	120°	128°	134°	116°	....	123°
α-allylphenylhydrazone	145°	155°	142°	157°	....	132°
α-benzoylphenylhydrazone	170°	165°	165°	154°	....	128°
di-phenylhydrazone	218°	161°	155°	157°	....	....
β-naphthylhydrazone	141°	....	157°	167°	176°	203°

OF OSAZONES

	Arabinose	Glucose	Mannose	Galactose	Maltose	Lactose
Phenylosazone	160°	208°	208°	193°	206°	200°
p-bromophenylosazone	196-200°	222°	.....	....	198°	....
p-nitrophenylosazone	.....	257°	.....	....	261°	258°

Glucosides

A glucoside is a compound which, upon hydrolysis with acids, yields glucose (or another sugar) and one or more other substances. A great variety of substances occur in plants, and to a lesser extent in animals, combined with a sugar (usually d-glucose). The general formula is



in which R may represent an alcohol, acid, aldehyde, phenol or a large number of other substances.

They are usually prepared by extraction with water or alcohol, and are mostly colorless, levorotatory, crystalline substances, with a bitter taste.

Most glucosides may be hydrolyzed by enzymes contained in the same tissue, but in other cells of the same plant from which the glucoside is obtained. These enzymes have the generic name of glucosidases. The best known glucosidase is emulsin of almonds. It hydrolyzes only β-glucosides, i. e., derivatives of β-glucose. Maltase hydrolyzes α-glucosides. These specific reactions have proven very useful in the elucidation of the structure of many glucosides and polysaccharides. Myrosin, obtained from black mustard seeds, is another enzyme of wide application. It acts upon many glucosides, all of which contain sulphur, such as glucotropaolin, sinalbin and sinigrin.

While d-glucose is found as a constituent of glucosides more often than all other sugars, many other sugars may be found in glucoside combination. Galactose is a constituent of a number of plant glucosides (solanin, digitonin, etc.) and of a group of substances found in nerve tissue, called galactosides or cerebrosides. d-Ribose also forms important glucosides, among which are the four nucleotides, which make up plant nucleic acids. Glucosides of d-arabinose and l-arabinose, l-xylose and a number of methyl pentoses are also known.

TABLE V

SOME OF THE NATURAL GLUCOSIDES *

	Glucoside	M. P.	Products of Hydrolysis
			Phenols
Arbutin	$C_{12}H_{16}O_7$	187°	Glucose + hydroquinone
Phlorhizin	$C_{21}H_{24}O_{10}$	170°	Glucose + phloretin
			Aldehydes
Amygdalin	$C_{20}H_{27}O_{11}N$	200°	2 Glucose + d-mandelonitrile
			Acids
Jalapin	$C_{34}H_{56}O_{16}$	131°	Glucose + jalapinolic acid
Strophantin	$C_{40}H_{66}O_{19}$	....	Rhamnose + mannose + strophantidin
			Mustard Oils
Glucotropaolin	$C_{11}H_{15}O_9NS_2K$	....	Glucose + benzyl isothiocyanate + $KHSO_4$
Sinalbin	$C_{30}H_{42}O_{15}N_2S_2$	138°	Glucose + sinapin acid sulphate + acrinyl-isothiocyanate
Sinigrin	$C_{10}H_{16}O_9NS_2K$	126°	Glucose + allyl isothiocyanate + $KHSO_4$
			Various
Digitalin	$C_{35}H_{56}O_{14}$	217°	Glucose + digitalose + digitaligenin
Digitonin	$C_{54}H_{92}O_{28}$	225°	Glucose + galactose + digitogenin
Digitoxin	$C_{31}H_{54}O_{11}$	145°	2 Digitoxose + digitoxigenin
Indican	$C_{14}H_{17}O_6N$	100°	Glucose + indoxyl
Saponarin	$C_{12}H_{14}O_7$	....	Glucose + saponaretin
Saponins	.....	....	Glucose + galactose + sapogenins
Vernin	$C_{10}H_{13}O_5N_5$	....	d-Ribose + guanine

* Arranged after R. F. Armstrong, The Simple Carbohydrates and Glucosides, Longmans, Green & Co., N. Y., 1912.

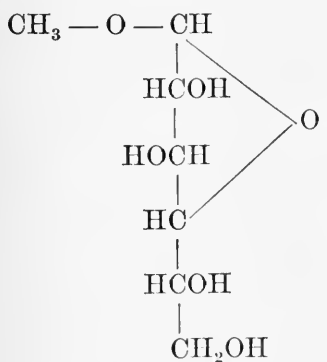
Special Properties of Monosaccharides.—The general properties and reactions of the monosaccharides have just been presented and it remains to point out properties of the individual carbohydrates that are of special interest biologically.

Hexoses.—Only two hexoses are found naturally as such, d-glucose and d-fructose; d-glucose, the most common monosaccharide occurring in nature, is found in most plant and animal tissues. Commercially it is obtained by hydrolyzing starch with dilute acid. This glucose is a mixture of α- and β-glucose and is called γ-glucose. It is readily purified by one crystallization from glacial acetic acid and washing with alcohol. From aqueous solution it crystallizes with one molecule of water. This form melts at 86° C. The anhydrous form, obtained by crystallization from aqueous solution at high temperature, melts at 146° C. One hundred parts of water dissolve 81.7 parts of anhydrous glucose at 15° C., while in alcohol it is rather insoluble. It is insoluble in ether and almost insoluble in acetone. Its aqueous solutions are neutral and are not electrolytes.

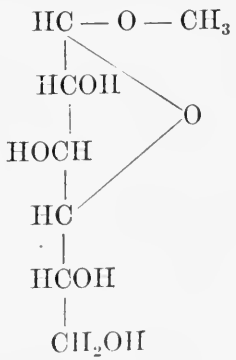
When heated to 170° it darkens and gives off much water, leaving in the residue a deliquescent substance, glucosan, which can be converted to glucose by boiling with water or acids. It is not sweet nor does it undergo fermentation. It is dextrorotatory.

Methyl Glucosides.—α-Methyl glucoside was first obtained by E. Fischer, by dissolving glucose in acetone-free anhydrous methyl alcohol, containing 0.25 per cent hydrogen chlorid, heating it under pressure, distilling off the alcohol and obtaining the crystals from the residual solution. Both the α- and β-methyl glucosides are found in this reaction, the equilibrated mixture containing 77 per cent of the α-form.

α-Methyl glucoside forms rhombic crystals melting at 165° C., easily soluble in water, difficultly soluble in cold alcohol, practically insoluble in ether. Its specific rotation is + 157° and does not show mutarotation. It does not reduce, does not form hydrazones, nor exhibit any aldehydic properties and is therefore believed to exist in the γ-lactone form only.



α-Methyl Glucoside



β-Methyl Glucoside

If the mother liquid from the methyl glucoside be concentrated to a syrup and allowed to stand for several weeks, β-methyl glucoside will crystallize out. It can be more readily obtained from this mother liquid by treating it with yeast, which hydrolyzes the α, but not the β-form, to glucose, and this in turn is converted to ethyl alcohol and carbon dioxid. β-methyl glucoside crystallizes with one half molecule of water of crystallization, and melts at 108° C. Its specific rotation is — 32°.

By boiling with acids both methyl glucosides are converted into glucose and methyl alcohol. α-Methyl glucoside is also hydrolyzed by maltase, an enzyme of yeast, but β-methyl glucoside is not. Emulsin, an enzyme found in bitter almonds, decomposes the β-methyl glucoside, but not the α-form. This is a splendid illustration of the specificity of biochemical reactions.

Mannose.—d-Mannose occurs free in some plants, but usually it is found as an anhydride condensation product called Mannan.[6] It is most readily prepared from the vegetable ivory nut by hydrolysis with dilute hydrochloric acid, neutralizing the acid and converting the mannose to the very insoluble, characteristic mannose hydrazone, from which mannose is obtained in the usual way. A not uncommon form in which mannose also occurs in nature is as the alcohol mannitol. Mannose can be obtained from mannitol by oxidation. This was the method by which it was first prepared (Fischer and Hirschberger) and only later was it identified with the natural product. On the other hand, d-mannitol may be prepared by reduction of d-mannose with sodium amalgam.

In general behavior, mannose is quite similar to d-glucose. It forms the same phenyl osazone, exhibits mutarotation and has similar solubilities. It forms rhombic crystals, melting at 132° C.

Galactose.—d-Galactose is rarely found free in nature. When found, it is often the result of special conditions. For example, Lippmann discovered galactose as a crystalline efflorescense in ivy berries after a sharp frost—the first of the autumn. Usually galactose occurs combined with sugars and with other substances as galactosides. It is most commonly found combined with glucose, as lactose in milk, and with sucrose in the trisaccharide raffinose, in beets.

$$\underset{\text{lactose}}{C_{12}H_{22}O_{11}} + H_2O \longrightarrow \underset{\text{d-glucose}}{C_6H_{12}O_6} + \underset{\text{d-galactose}}{C_6H_{12}O_6}$$

$$\underset{\text{Raffinose}}{C_{18}H_{32}O_{16}} + 2H_2O \longrightarrow \underset{\text{d-Fructose}}{C_6H_{12}O_6} + \underset{\text{d-Galactose}}{C_6H_{12}O_6} + \underset{\text{d-Glucose}}{C_6H_{12}O_6}$$

It is interesting to observe that the amount of raffinose found in the beet is increased when the plant is subjected to a sudden frost.

From algæ, lichens and mosses, mucilages can be obtained that yield

[6] Polymers of the sugars are given the name of the sugar with the ending—an. Thus common starch is a glucosan.

galactose on hydrolysis. Galactose is present here in a polymeric form called galactans. Galactans are also found in certain gums and pectins. The pectins are found in apples, pears, beets, carrots, flax, etc., and these, on mild hydrolysis, are converted to pectic acids, the calcium salts of which cause fruit juices to jell. On hydrolysis with acids they yield d-galactose and l-arabinose.

It is usually prepared from lactose by heating with two per cent sulphuric acid, precipitating the sulphuric acid with barium carbonate and concentrating the filtrate to a syrup from which d-galactose slowly crystallizes in large prisms with one molecule of water of crystallization. The hydrated form melts at 118-120° C. From alcoholic solution it crystallizes in leaflets which melt at about 165° C. It is sweet, easily soluble in water, but practically insoluble in absolute alcohol and in ether. It behaves somewhat like d-glucose; it exhibits mutarotation, both α- and β-forms having been prepared and when treated with sodium amalgam, it is reduced to the alcohol dulcitol, which occurs naturally in Madagascar manna.

On oxidation with nitric acid mucic acid is formed. Mucic acid is a very characteristic oxidation product of galactose (and lactose), with a melting point of 212-215° C., quite insoluble in water (about 0.3 per cent at 15° C.), and therefore is used frequently as a means of identifying galactose. It is optically inactive.

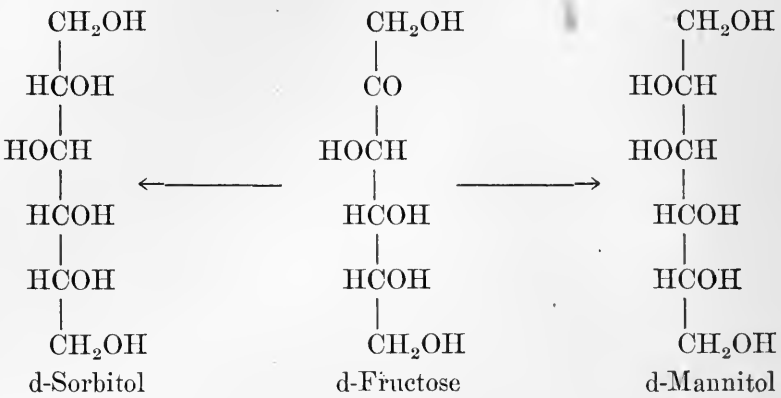
Fructose.—d-Fructose (levulose) was discovered by Dubrunfaut in 1847 in the hydrolysis products of cane sugar. It occurs in the juices of many plants and fruits with glucose, especially in tomatoes, certain manna and mangoes. In young sugar cane it occurs in equal amount with glucose and sucrose. As the cane grows older, the proportion of fructose to the two other sugars decreases to about 15 per cent and in the mature plant to about 1.5 per cent of the total amount of the three sugars present. In honey, glucose and fructose are found in nearly equal proportions, together with a little sucrose and dextrine.

d-Fructose also occurs combined with other sugars, as in sucrose (glucose and fructose); raffinose (glucose, galactose and fructose); etc. It is a constituent of certain glucosides and saponins. The polysaccharide inulin, which is obtained in quantity from the tubers of the dahlia, sunflower and other members of the same family, is a fructosan, and hence yields only fructose on hydrolysis. This is, in fact, the simplest way to obtain fructose, as from 7 to 17 per cent of inulin is found in the roots of the dahlia. It is purified by recrystallization from water at 60-70° C.

Fructose forms anhydrous rhombic crystals, tastes almost as sweet as cane sugar and melts between 95 to 105° C. It is very soluble in water and hot alcohol, but only slightly soluble in cold alcohol. Its aqueous solutions exhibit the property of mutarotation and exist in solution, presumably as an equilibrated mixture of stereoisomeric forms, but the two forms

have not yet been separated, as have the two forms of glucose and other sugars.

Fructose is reduced by sodium amalgam to two alcohols, d-mannitol and d-sorbitol being formed in equal quantities.



By oxidation with mercuric oxid, for example, fructose is converted to acids having less than six carbon atoms, such as carbonic, formic, glycollic, oxalic, tartaric and d-erythronic acids. When boiled with dilute mineral acids, it forms levulinic acid ($CH_3 — CO — CH_2 — CH_2 — COOH$), formic acid and other substances. Levulinic acid is a characteristic degradation product of hexoses and hexosans, and is used as a means of differentiating between hexoses and pentoses.

Levulinic acid is a colorless oil that boils at 146° C. at 18 mm. pressure. It crystallizes in rhombic leaflets when placed over sulphuric acid in a cool place. The crystals are deliquescent, easily soluble in water, alcohol and ether, and melt at 33° C.

Pentoses.—Eight aldopentoses are theoretically possible, and of these seven are known. Pentoses exhibit mutarotation, and therefore, like the hexoses, indicating that they exist in an α and β and γ lactone form. Two of them, arabinose and xylose, are widely distributed in the vegetable world as polysaccharides, called pentosans. They are very resistant to the action of alkali and are hydrolyzed by dilute acids to form the simple sugars:

$$\underset{\text{Pentosan}}{(C_5H_8O_4)_n} + (H_2O)_n \longrightarrow \underset{\text{Pentose}}{(C_5H_{10}O_5)_n}$$

Pentoses are distinguished from hexoses by their behavior when boiled for a long time with hydrochloric acid. Hexoses are converted to levulinic acid by this treatment, while pentoses form furfuraldehyde. Pentoses may be estimated by the use of this reaction. The furfuraldehyde is distilled off and then coupled with phloroglucinol and the condensation product is weighed.

TABLE VI—ALDOPENTOSES

The same reaction is used for the qualitative detection of pentoses. Color reactions are obtained by heating pentose with hydrochloric acid in the presence of phloroglucinol or orcinol.

Xylose.—l-Xylose (wood sugar) is formed from the xylans called wood gums, found in vegetable cell walls, and next to cellulose the most important carbohydrate found in plants. It forms monoclinic prisms or needles; has a sweet taste, is readily soluble in water and hot alcohol, but not in ether. It melts at 135° according to some, as high as 154°, according to others. Its specific rotation is + 85.7°. The equilibrated mixture has a specific rotation of + 18.5°.

It gives the usual aldose reactions. It is best identified by oxidizing to l-xylonic acid and converting the latter to the characteristic double cadmium bromid salt.

$$(C_5H_9O_6)_2.Cd.CdBr_2.2H_2O$$

l-Arabinose.—This pentose was first isolated by Scheibler (1873). The gums of cherry, plum, gum arabic, etc., are composed chiefly of arabans, and from them l-arabinose is obtained on hydrolysis with acids.

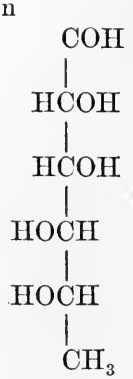
It crystallizes in needles, melting at 160° C. It is readily soluble in water, difficultly soluble in 95 per cent alcohol and almost insoluble in

absolute alcohol. It exhibits strong mutarotation in aqueous solution. The specific rotations for α-l-arabinose, β-l-arabinose and the equilibrated mixture are + 76°, +184° and + 104° respectively.

The most characteristic compounds of arabinose are parabromophenyl hydrazone, diphenyl hydrazone and phenyl-osazone. The diphenyl hydrazone, melting at 218° C., is a colorless crystalline substance and is usually used for identifying arabinose.

d-Ribose.—Unlike the other two pentoses which have been considered, d-ribose does not appear as a pentosan, but is an important constituent of plant nucleic acid, as proven by Levene and Jacobs (1912). It seems probable that the known plant nucleic acids are quite similar, and it has been established that there are four molecules of d-ribose in those plant nucleic acids that are known.

Methyl Pentoses.—Several of these have recently been isolated from plants. They differ from pentoses in having a methyl radical replace one of the hydrogens of the primary alcohol, — CH_2OH, forming the group $CHOH.CH_3$, as in



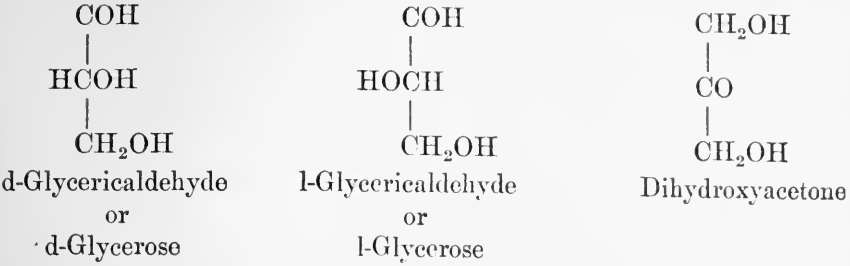
Rhamnose.—l-Rhamnose is a constituent of many glucosides and is perhaps the most common of the methyl pentoses. It crystallizes with one molecule of water and exists in α and β forms.

Digitoxose is probably a reduced methyl pentose obtained from digitalis:

$$CH_3.CHOH.CHOH.CHOH.CH_2.COH$$

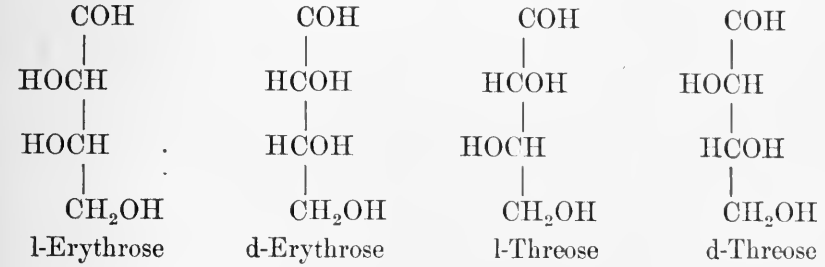
The methyl pentoses behave like the pentoses on the whole, but yield methylfurfuraldehyde on distillation with acids.

Dioses, Trioses, Tetroses, etc.—The simplest sugar is the diose, glycolic-aldehyde, $\begin{matrix} COH \\ | \\ CH_2OH \end{matrix}$ but it has not been found in nature. It is of interest, however, as a possible product of the intermediary metabolism of carbohydrates. There are three trioses of interest, two aldoses, d-and l-glyceroses or glycericaldehydes, and one ketose, dihydroxyacetone.



All of these substances are intermediary products in the metabolism of carbohydrates, and are of interest on that account.

There are four possible aldotetroses of which three are known, but they have not been found to occur in nature in the free states.



The alcohol of erythrose, erythritol, has been obtained from various algæ and mosses.

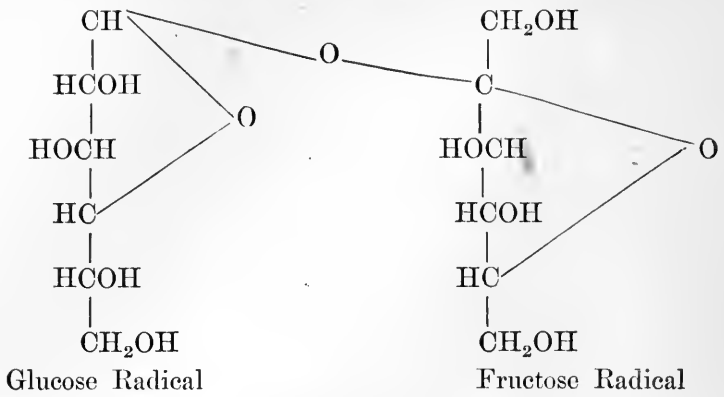
Disaccharides

These sugars contain twelve carbon atoms and are made up of two hexoses united by an oxygen atom. When acted upon by hydrolytic agents, they take up one molecule of water and are converted into the hexoses of which they are composed.

The hexoses in these carbohydrates are bound together in much the same way as they are in the glucosides; hence the aldehyde or ketone radical of one of the hexoses is the point of union, while the ketone or aldehyde radical of the other hexose may or may not remain free.

Those disaccharides that have a potentially free aldehyde or ketonic group give the typical reactions of the hexoses, such as reduction of alkaline copper and other metallic hydroxides and combination with hydrocyanic acid. They exhibit mutarotation and exist in two forms which are in equilibrium in aqueous solution. The union of the two hexoses is similar to that found in the case of the glucosides. In fact, many of them are hydrolyzed by certain glucosidases.

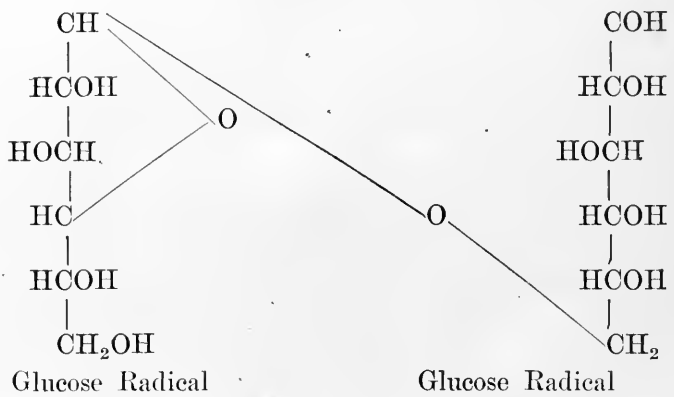
When an aldehyde or ketone group is free, as in maltose, phenyl osazones, that are only slightly soluble but difficult to purify, are obtained. The hydrazones are almost all easily soluble in water. The disaccharides



Glucose Radical — Fructose Radical

Sucrose or Cane Sugar

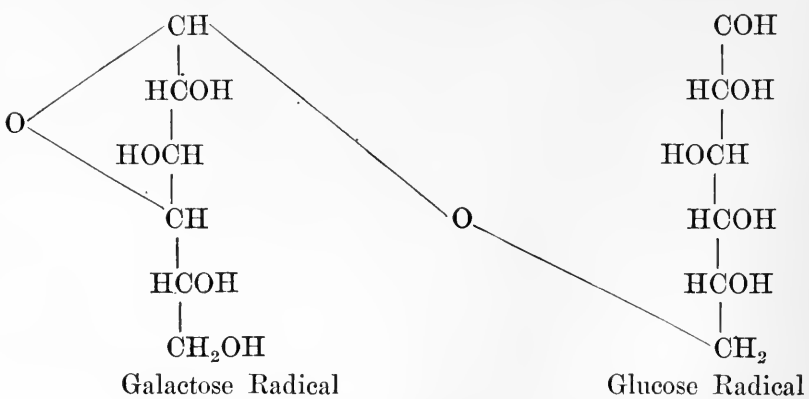
(Neither aldehyde nor ketone functional)



Glucose Radical — Glucose Radical

Maltose

(One aldehyde radical free and functional)



Galactose Radical — Glucose Radical

Lactose

(One aldehyde radical free and functional)

that have no free aldehyde or ketone do not form osazones. Other than the phenyl osazones, the disaccharides form no compounds that are characteristic.

In the determination of the configuration of the disaccharides, the chief points to be elucidated were (1) the nature of the component hexoses, (2) whether the disaccharide was an α- or β-glucoside, (3) the place of union of the two monosaccharides.

The nature of the component hexoses was determined by hydrolyzing the disaccharide with acid and identifying the hexoses. The nature of the glucosidic union was established by the behavior of the disaccharide toward maltose and emulsin. If the disaccharide is hydrolyzed by maltase, it is an α-glucoside; if by emulsin, it is a β-glucoside. This point has also been determined by studying the optical behavior of the hexoses as soon as formed by the action of an enzyme, toward a drop of alkali. If the rotation is increased, it indicates the presence of a β-glucose; if the mutarotation is in the other direction, an α-glucose has been formed.

Points of special interest of the individual disaccharides will now be presented.

Sucrose.—Sucrose, known also as saccharose or cane sugar, is industrially the most important of the disaccharides. It is very widely distributed in the plant world, where it serves chiefly as a reserve material.

It crystallizes readily, is very soluble in water and very sweet. It does not exhibit mutarotation in aqueous solution. It is dextrarotary and has a specific rotation of + 66.5°. When heated, it melts at 160° C., and at 200° C. it darkens, forming caramel, in which process water is given off.

Chemically, sucrose behaves neither as an aldehyde nor as a ketone; it does not form hydrazones or osazones, nor does it reduce Fehling's solution. Sucrose is readily hydrolyzed by boiling with acids, one molecule of glucose and one of fructose being formed. The same hydrolysis may be brought about by an enzyme, invertase or sucrase, present in yeasts and other fungi, as well as in many other plants and in the digestive tracts of many animals.

The products of hydrolysis of sucrose have a resultant levorotation, since fructose is more levorotatory than glucose is dextrorotatory. This process is therefore called inversion and the product invert sugar. Because sucrose exhibits neither aldehyde nor ketone properties, it is believed that the glucose and fructose molecules, that compose the sucrose molecule, are united in such a way that both aldehyde and ketone groups are destroyed. The formula usually ascribed to sucrose is Fischer's modification of the Tollens formula, in which it is both a glucoside and a fructoside.

Lactose.—Lactose or milk sugar was first obtained about 1615 by Fabricio Bartoletti. It is always found in the mammary secretion, but has not been found in the vegetable kingdom. It is often found in the

urine of pregnant and lactating women. Human milk contains 5 to 7 per cent lactose, occasionally more, while the milk of other animals contains somewhat less.

Lactose is readily prepared from milk by coagulation of the casein with the enzyme rennet, and the clear liquid or whey which separates from the precipitated protein is concentrated under diminished pressure to a syrup, from which crude lactose crystallizes. It is purified by recrystallization from water.

Erdmann (1855) obtained lactose in two crystalline forms, one of which had a specific rotation of + 90° and the other of + 35°, each showing a motarotation and the specific rotation of the equilibrated solution being + 55.3°. This was the first of the disaccharides in which the existence of more than one form was demonstrated.

Sodium amalgam reduces lactose, forming mannitol, dulcitol, lactic acid, hexyl-alcohol and other products. Lactose is a glucose-galactoside and not a galactose-glucoside, as shown by its behavior on gentle oxidation, so that only the free aldehyde group will be oxidized. Under such conditions lactobionic acid is formed, which on hydrolysis yields galactose and gluconic acid, showing that the free aldehyde group is that of glucose, while, if the free aldehyde group were that of galactose, galactonic acid and glucose would result from the hydrolysis of the oxidation product.

Lactose is much more difficultly hydrolyzed by acids than sucrose. It is also hydrolyzed by the enzyme lactase, found in the intestinal mucose of animals, as well as by aqueous extracts of kefir and some yeasts and almonds (crude emulsin). It is not hydrolyzed by maltase, invertase or any enzymes in brewers' yeast. This serves as a simple means of distinguishing between lactose and glucose, a problem often met with by the pathological chemist, since glucose is readily fermented by yeast. Lactose also forms a fairly characteristic osazone, which may be readily distinguished from glucosazone. A good way to prepare the osazones from biological material is to precipitate most of the interfering substances by adding mercuric nitrate in dilute nitric acid solution and then solid sodium carbonate. Then filter, cover the filtrate and prepare the osazone in the usual way with phenylhydrazine hydrochlorid and sodium nitrate.

Maltose.—Maltose or malt sugar is formed by the action of diastase upon starch. The sugar was first isolated by De Saussure in 1819, but its identity was determined by Debrunfaut in 1847 and he gave it the name maltose. It occurs in plants and animal tissues to some extent, and results from the action of diastase of the pancreatic secretion, or ptyalin of saliva on starch or glycogen.

Maltose crystallizes in small needles with one molecule of water of crystallization. It is easily soluble in water and in alcohol its solubility is 5 per cent. Its solutions show mutarotation. Its specific rotation initially is + 119° and that of the equilibrated mixture is + 137°.

Maltose reduces Fehling's solution and forms a phenyl osazone. It is hydrolyzed by acids forming two molecules of glucose, but is more resistant to hydrolysis than sucrose. Maltose is also hydrolyzed by maltase in the same way, but is not hydrolyzed by emulsin. Because of this behavior, maltose is assumed to be a glucose-α-glucoside.

Polysaccharides

Those considered under this heading form colloidal solutions or are insoluble in water. The more important ones are starch, glycogen, cellulose, dextrins, inulin and gums. They are usually named from the sugar they yield on hydrolysis, with the suffix "an." Thus starch is a glucosan; inulin is a levulan.

Starch is one of the polysaccharides found in plants in the form of a granule with a characteristic structure, so that it is possible to identify the plant from which the starch came by microscopic examination. It forms the reserve food of the plant cell. It is insoluble in the ordinary solvents, but if poured into boiling water the granule is disrupted and a colloidal solution results.

Upon hydrolysis with acids or enzymes, a series of simple polysaccharides are formed, namely, soluble starch, erythrodextrin, achroödextrin, and finally, maltose and glucose. It has been quite difficult to obtain any knowledge of the number of hexose groups in starch and the dextrins.

Inulin is a levulan, found in the tubers of the dahlia and Jerusalem artichokes. It forms the best source of obtaining d-levulose. It is not unlike starch in its chemical behavior.

Cellulose is the main constituent of the wall of plant cells. It has a more complex structure than starch. It is insoluble in all the usual solvents, but will dissolve in ammoniacal copper salt solutions. On hydrolysis with acids it yields glucose and other monosaccharides. Nitric acid with cellulose forms nitrocellulose or gun cotton. Concentrated sulphuric acid dissolves cellulose. Upon diluting with water, it is again precipitated, but in a different form. The resulting compound gives a blue color with iodin and is called amyloid.

A number of cellulose-like substances, called hemi-celluloses, are found in seeds and young plant tissues. They probably act both as supporting structures and as a source of reserve food. Upon acid hydrolysis they yield galactose, arabinose, mannose, rhamnose and occasionally fructose.

Gums are usually pentosans. They are white substances which dissolve in water, giving a thick, viscid, mucillaginous solution. Examples are gum acacia (or arabic) and gum tragacanth. Upon hydrolysis they yield pentoses or their derivatives, such as arabinose and rhamnose. Occasionally hexoses also result from hydrolysis of some gums, such as man-

nose and glucose. Phosphoric acid is usually associated with the gums, as with many other polysaccharides, and it is most difficult if not impossible to separate them. This suggests that sugar phophate may be present in the polysaccharide molecule. Phosphoric acid sugar compounds play a great rôle in biochemical phenomena.

Digestion of Carbohydrates

The carbohydrates that play a rôle in human metabolism are the polysaccharides, starches, glycogen and cellulose, and the disaccharides, sucrose, lactose and maltose. During the process of digestion, the higher carbohydrates are converted into monosaccharides, by processes of hydrolysis.

Salivary Digestion.—The first enzyme that acts upon carbohydrates is encountered in the salivary secretion and is known under the names of amylolytic ferment, diastase and ptyalin. It is a ferment that is susceptible to changes in temperature. At 0° C. its activity is entirely suspended, whereas at body temperature it shows its optimum activity. If the temperature is raised above that, its activity diminishes until it reaches 65° to 70° C., when it is completely destroyed.

It is also highly sensitive to the hydrogen ion concentration, showing greatest activity in an acid concentration of $\frac{N}{10000}$. An acid solution of $\frac{N}{100}$ inhibits the action of the diastase completely, as will also a strongly alkaline reaction.

Salts, especially phosphates, seem necessary for ptyalin digestion for, when saliva is dialyzed, it loses much of its amylolytic powers. These may be restored by the addition of a little phosphate. It is quite possible that a carbohydrate-phosphate intermediary product of digestion is formed similar to the hexose-phosphate that Harden and Young found to be essential in fermentation. Salts of the heavy metals—such as uranium, silver and mercury—will severely inhibit the action of ptyalin.

During the process of mastication the food is brought into intimate contact with the saliva, but does not have sufficient time to bring about considerable digestion. The greatest activity of ptyalin takes place in the fundus of the stomach, before the acidity of the stomach reaches the level of concentration at which it inhibits the action of ptyalin.

Action of Ptyalin.—The ptyalin does not affect cellulose. It acts on boiled starch much more readily than on native starch. It acts by bringing about a process of hydrolysis whereby the large starch molecule, which belongs to the suspension colloidal group, is broken up into smaller and smaller molecules, passing through various stages of "colloidality," be-

coming a soluble starch, then going through various stages of dextrins, until it finally reaches the stage of the perfectly soluble disaccharide, maltose.

It is impossible at present to sharply separate the different intermediary products in starch digestion. The different stages, however, can be recognized by means of the iodin reaction. The native starches give a blue coloration with iodin, and as digestion progresses dextrins are formed which give at first a violet red, then brown red, and finally no color reaction at all with iodin. These dextrins are known respectively as erythrodextrins and achroödextrins.

In the salivary secretion we find another enzyme which acts on maltose and is known as maltase. It acts on the maltose molecule, making it undergo hydrolysis, and converting it into two molecules of glucose.

Gastric Digestion of Carbohydrates.—In the gastric secretion there are no enzymes which attack carbohydrates. As long as the acidity of the gastric contents is low the ptyalin and maltase, which are swallowed with the saliva, may continue their activity. When the gastric acidity increases in concentration it may help in hydrolyzing the disaccharides, but this takes place only to an insignificant extent.

Intestinal Digestion of Carbohydrates.—In the pancreatic secretions we find an amylolytic enzyme which has all the properties of ptyalin, but which has the power of acting at a much greater velocity. The intestinal juices also contain three enzymes: sucrase, which has the power of splitting sucrose into glucose and levulose; maltase, which splits maltose into two molecules of glucose, and lactase, which splits lactose into glucose and galactose. All the carbohydrates, therefore, are brought down in the intestinal canal to the stage of monosaccharides. Separate enzymes are present there for all types of carbohydrates that the human individual ingests, except cellulose, which is left entirely untouched, and is eliminated as such.

Absorption of Carbohydrates

The products of carbohydrate digestion are very soluble and easily diffusible. The amount that is absorbed by the stomach is very small and of no practical consequence. Practically all of the digested carbohydrates are absorbed in the small intestines and very little is left in the material that reaches the ileocecal valve.

All the absorbed carbohydrates are carried away by the blood stream into the portal vein, thence to the liver. It is remarkable that in spite of the easy solubility of sucrose and lactose, none of it is absorbed under ordinary circumstances. The intestinal wall is almost impermeable to them, whereas maltose may be absorbed to a slight extent. The body cells have the power of utilizing maltose, probably because of the presence of a maltase in the blood stream, but cannot utilize sucrose or lactose;

and if these enter the blood stream parenterally, they are quantitatively excreted in the urine.

The carbohydrates that are absorbable, therefore, are the three monosaccharides—glucose, levulose, galactose—and the one disaccharide—maltose.

The Sugar of the Blood.—That glucose is the most important sugar of the blood we know definitely. Whether levulose and galactose exist in the blood as such is at present not known. From the ease with which these two sugars are converted into glucose when fed to a diabetic individual, we have every reason to believe that they are converted into glucose either in the process of absorption or soon thereafter.

Glucose exists in the blood in a state of free solution and not in any chemical union. (Michaelis and Rona (1908).)

When one examines the blood of an individual for its glucose concentration at frequent intervals of time, one finds that under normal conditions it fluctuates within surprisingly narrow limits. In the morning before breakfast, it usually is at its lowest level, between 0.07 to 0.10 per cent. Between one and one and a half hours after a meal rich in carbohydrates, it rises to a level of 0.10 to 0.15 per cent. After that it gradually comes down, to reach the fasting level about two to three hours after the meal. This cycle of events repeats itself with each meal.

If a normal individual is allowed to fast for some time, the blood sugar remains about 0.07 per cent and very seldom sinks below that figure. In such cases there is hardly any fluctuation in the blood sugar concentration from hour to hour.

There are a number of forces which are operative in keeping the blood sugar concentration at such a constant level, and these are: I. Those that prevent it from rising above normal levels; II. Those that prevent it from falling below normal levels.

The factors that prevent the blood sugar from rising above normal levels are: 1. Polymerization of glucose into glycogen by the cells of the liver and muscles; 2. Utilization of glucose (oxidation) by the cells of the body for dynamogenetic purposes; 3. Conversion of glucose into fat.

The factors that prevent the blood sugar concentration from falling below normal levels are: 1. Mobilization of glycogen from its storehouses —liver and muscle—and its hydrolysis, which results in glucose formation; 2. Increase in protein metabolism with the result that a large number of amino acids are converted into glucose.

The moment sugar enters the intestinal canal its absorption begins. This causes an increase in the glucose concentration of the blood in the portal vein. Synchronous with the increase in the portal concentration, there takes place a withdrawal of glucose from the blood by the liver cells and their polymerization of the glucose into glycogen. On the other hand, when absorption of carbohydrates from the intestinal canal has stopped, the

venous blood becomes poorer in glucose. The process then reverses. The glycogen in the liver cells becomes hydrolyzed and a stream of glucose starts into the blood. Apparently there must exist a very delicately adjusted physicochemical relationship between the glucose concentration of the portal blood, the glycogen content of the liver, and the glucose concentration of the hepatic vessels.

The capacity of the liver to store glycogen is enormous. Schoendorf (1903 (*b*)) showed that the liver of dogs may contain as much as 18.7 per cent of glycogen, and Otto (1891) showed that rabbit's liver may contain as much as 16.8 per cent of glycogen after ingestion of large amounts of carbohydrates. The liver of a man weighing about 70 kilos weighs approximately 2000 grams. On the basis of the above figures, we can readily see that it can hold as much as 300 grams of glycogen, which is considerably more carbohydrate than the average man consumes in any one meal.

The liver, therefore, through its glycogenetic function acts as a wonderful regulator of the sugar in the blood. It prevents any marked fluctuations in the concentration, and above all, any sudden increases in the sugar content, which would be followed by loss of sugar through glucosuria.

The utilization of glucose by the muscle cells occurs as soon as its absorption from the intestinal canal begins (Lusk, 1912-1915). Apparently the body cells burn glucose with greater ease than any other foodstuff, for, when glucose is present in abundance, the combustion of fat is stopped almost completely, and that of protein is reduced to an absolute minimum. Glucose in the body burns to CO_2 and H_2O, according to the following reaction:

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$

From this we see that when glucose is oxidized a certain volume of oxygen is required, and for every volume of oxygen used, a corresponding volume of carbon dioxid is given off. The ratio between the volumes of CO_2 and O_2 is known as the Respiratory Quotient. The value of $\frac{CO_2}{O_2}$ in this case equals 1. In the combustion of no other foodstuff does the Respiratory Quotient equal 1. When fat burns the $\frac{CO_2}{O_2}$ quotient is 0.707, and when protein burns, the quotient is 0.801.

In Lusk's experiments on dogs, forty-five minutes after glucose ingestion, the respiratory quotient was 0.99, showing that glucose burnt almost exclusively.

If the absorption of glucose from the intestinal canal still continues, we have a third factor brought into play, namely its conversion into fat.

In normal individuals, during the process of glucose absorption from the intestinal canal, we have a series of three outlets which are operating to prevent its accumulation in the blood. Schematically we may repre-

sent the arrangement by an inclined tube that has a series of outlets at different levels, with openings at the bottom through which sugar may be pumped in. The level of sugar in this inclined tube will depend upon the speed with which it is pumped in and with which it pours out at the various outlets. If the inflow is so rapid that the first outlet cannot take care of it all, it will mount until it reaches the second. If that is not sufficient, it will reach the third, and if that is not sufficient, it will mount still higher.

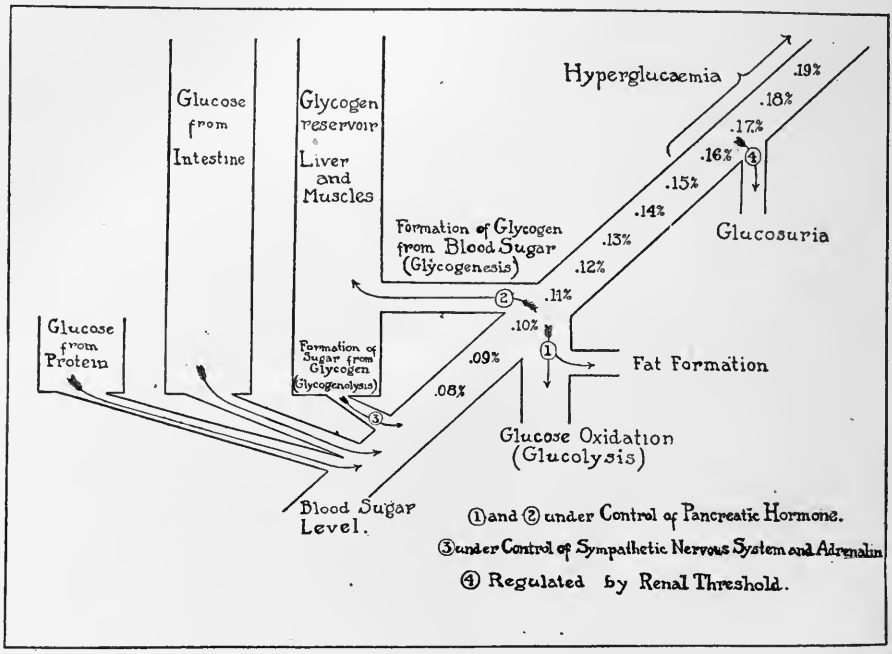


Fig. 2. Schematic illustration of the factors which regulate the sugar concentration of the blood.

The level of sugar in this tube at any given time will depend upon the relationship between the velocity and volume of the sugar inflow at the bottom, and the volume and velocity of its outflow through the three normal channels.

In the body, the glucose concentration of the blood at any given time also depends upon the speed and amount of its absorption from the intestinal canal, and upon the speed of its removal by utilization, glycogen and fat formations. Normally it seldom goes above 0.12 or 0.13 per cent, because the glycogen formation proceeds at such a rapid pace that it does not permit its accumulation in the blood. When we ingest carbohydrates in the form of starch, we can take absolutely unlimited quantities. Because the digestion of it is rather slow, the absorption follows suit, and

at no time do we find an accumulation above those levels. If, however, we ingest a large amount of carbohydrates in the form of glucose which requires no digestion at all, and which is absorbed with great rapidity, we find that glucose enters the blood stream at such a rapid pace that the three outlets—utilization, glycogen formation, fat formation—are not sufficient to remove it all. Its concentration in the blood stream rises and we develop what is known as a condition of *hyperglucemia.*

Another process may be brought into play at this stage, namely that of *glucosuria.*

It is a well-known fact that the kidneys exercise a selective action on the substances that circulate through it in the blood stream. At the present state of our physicochemical knowledge it is difficult to say what the mechanism of kidney secretion is. But we do know that for a number of crystalloids the rate and amount of their excretion bears a definite relationship to their concentration in the blood. (Ambard and Weil, 1914; McClean, F. C., 1915.)

The behavior of glucose in the blood is like that of a pure crystalloid (Michaelis and Rona, 1908), and one would expect the kidneys to permit its free secretion in the urine. This, however, is not the case. With the ordinary reduction tests (Fehling's solution, Benedict's solution, etc.) we cannot detect the presence of glucose in the urine of normal individuals [7] if the blood sugar concentration fluctuates within the normal limits. When, however, the concentration of glucose in the blood rises, there comes a point at which the kidneys begin to excrete it in easily detectible quantities.

The height of blood sugar concentration at which the kidneys begin to secrete sugar differs with different individuals and is known as the *kidney threshold* for sugar. With a very few it lies as low as 0.08 per cent, which means that these people excrete glucose in detectible quantities all the time, and they suffer from a condition that is recognized as *renal glucosuria.* Others will not excrete it even when the concentration is as high as 0.26 per cent, as in cases of chronic nephritis. These two extremes are comparatively rare. The great majority of normal individuals, however, excrete glucose in the urine in detectible quantities when the glucose concentration of the blood rises above 0.15 to 0.16 per cent. There is at present no explanation for this individual variation, except for the statement that there must exist a difference in sensitiveness for glucose in

[7] Stanley R. Benedict has recently reported (1918) that the urine of a normal dog and of two normal men can be shown to contain substances which are fermentible by yeast and which reduce pieric acid. He assumes that it is glucose. The dog weighing 18 kilos excreted in the neighborhood of 390 mgs. per 24 hours when kept on an ordinary carbohydrate diet; 281 mgs. when kept on a low carbohydrate diet; 194 mgs. when fasting. His human subject, E. O., weighing 86 kilos, excreted 996 mgs. per 24 hours when on an ordinary carhohydrate diet; 777 mgs. when on a low carbohydrate diet; 1479 mgs. when on a carbohydrate-rich diet. The second subject, weighing 57 kilos, excreted 640 mgs. when on an ordinary diet; 543 mgs. when on a low carbohydrate diet; 847, 1156 and 1528 mgs. on each of three days of carbohydrate diet.

the kidney cells of different individuals. Because this glucosuria is caused by too rapid absorption of glucose from the alimentary canal, it is known as *alimentary glucosuria.*

Carbohydrate Tolerance. —In the preceding chapters it was shown that the body is capable of taking care of large quantities of carbohydrates (glucose) 1, by storing it in the cells of the liver and muscles in the form of a colloidal state—glycogen; 2, by utilizing, i. e., oxidizing it in preference to other foodstuffs; 3, by converting it into fat. It was further shown that these three factors tended to prevent the glucose from accumulating in the blood above certain concentrations, at which it surpasses the kidney threshold and forces the kidney cells to excrete the glucose in the urine.

The appearance of glucose in the urine in detectible quantities by means of the ordinary reagents (Benedict's or Fehling's solutions) has always been considered a sign that the individual has overtaxed the "carbohydrate tolerating" mechanism, and the amount of carbohydrate that it takes to bring about this condition has been known as the limit of his tolerance.

We shall see later that there are a number of pathological conditions which affect the carbohydrate tolerance of individuals and that the carbohydrate tolerance is therefore utilized as a means of detecting these pathological conditions. It is therefore of the utmost importance to have a clear concept of all the factors that determine and that may influence the carbohydrate tolerance of perfectly normal people.

In the light of our present knowledge that glucosuria is the result of hyperglucemia and that there exists a difference in the sensitiveness of the kidneys of different individuals to glucose concentration in the blood, it is advisable to eliminate this variable factor, and to determine the tolerance for carbohydrate on the basis of the blood sugar concentration. We would therefore define the *carbohydrate tolerance of an individual as that amount of carbohydrates (glucose)*[8] *which the individual can ingest without developing hyperglucemia,* and is in reality a test for the promptness with which the individual can convert glucose into glycogen and fat and also oxidize it.

Of course, one should not imply from the above that urinary examination for sugar is not necessary. It frequently does give valuable information.

Soon after the introduction of reliable methods for blood sugar determination (Lewis-Benedict, Bang) a whole series of studies were published on the blood sugar curves after the ingestion of variable amounts of glucose (Hamman and Hirschman, 1917. Hopkins, 1915. Jacobson, 1913. Bailey, 1919). The most satisfactory results are obtained after

[8] Glucose is used because this requires no time for digestion and thus another possibly variable factor is eliminated.

administering 100 grams of glucose dissolved in 400 c.c. of water to which has been added the extract 1 or 1½ lemons. This is to be taken in the morning before breakfast. The blood is examined for sugar immediately before the test meal, and at intervals of half hours after the meal, until the blood sugar comes back to normal.

With this procedure it is found that most subjects have an initial fasting blood sugar of 0.07 to 0.10 per cent; that about one hour after the ingestion of the glucose the blood sugar reaches the highest point, which is usually about 0.15 per cent or below; by the end of the second hour, it is well on the way to normal again.

If the individual's blood sugar rises above the level of 0.15 at any time after the ingestion of 100 grams of glucose, we are justified in concluding that he has interference with his carbohydrate tolerance. A number of records have been published on individuals classed as normal who show a much higher blood sugar concentration one hour after glucose ingestion. Future observations on the same individuals will reveal whether or not they were normal.

Carbohydrate Tolerance Standard.—It is of no practical value to know the maximum glucose tolerance of a person. But it is of great practical importance to know that by far the great majority of hundreds of cases of normal individuals who have received 100 grams of glucose have been able to tolerate it, i. e., have shown no hyperglucemia and no glucosuria when tested with the ordinary reagents.

The setting of any physiological standard is difficult. We have, for example, standard tables of weights. Are they in reality tables of what we do weigh or of what we should weigh? How many perfectly normal human individuals actually bear the exact height to weight ratio? Still we have accepted them as definite standards, realizing, of course, that we may have plus or minus variations from the theoretical without being classed as abnormal.

The study of the carbohydrate tolerance of human individuals is of comparatively recent development. And it will advance our science materially if those workers who reported hyperglucemias in what appeared to be normal individuals will repeat their tests on the same individuals at intervals of several years to see whether those people do not ultimately develop glucosuria and diabetes.

For persons weighing 60 kilos or more 100 grams of glucose should be given. For those weighing less, the amount should be reduced proportionately. But under no circumstances should more than 100 grams be given to people weighing more than 60 kilos, because the increase in weight is not so much due to muscle and liver (the glycogenetic organs) as to fat and skeleton which play no rôle in carbohydrate tolerance.

Glycogenesis and Carbohydrate Tolerance.—While we have three outlets for the stabilization of the blood sugar concentrations, the most im-

portant one, because of its enormous elasticity, is the glycogenetic function. It may truly be classed as a sort of "shock absorber" in the carbohydrate metabolism. The capacity of the liver for glycogen may reach 300 grams, while the muscles may hold as much as four per cent of their weight.

Glucolysis and Carbohydrate Tolerance.—The amount of glucose oxidation that can go on during a period of glucose plethora (as after ingestion of large amounts of glucose) is comparatively fixed and limited by the body's requirement for energy. Under those conditions no fat is burned and the utilization of protein is reduced to the "wear and tear" quota, which, from the dynamogenetic point of view, is insignificant. A man weighing 70 kilos will, when at rest, require approximately 35 calories per kilo per 24 hours. That means $70 \times 35 = 2450$ calories per 24 hours or 102 calories per hour. If all that were to come from glucose the maximum amount of glucose that he could utilize, i. e., oxidize, would be $\frac{102}{3.7} = 27$ grams per hour (each gram of glucose yields 3.7 calories), or for the two hours in which the carbohydrate tolerance test is made a maximum of 54 grams of glucose can be burnt. Fully half of the quantity given with a 100 gram test can be taken care of by oxidation.

The amount that can be taken care of by fat formation we do not know. It can be determined by studying the respiratory quotient (Lusk, 1912), but has not been worked out for man after a 100 gram glucose ingestion.

TABLE VII

TYPICAL BLOOD SUGAR CURVES OF NORMAL INDIVIDUALS *

M. McN. Healthy medical student, aged 24. Original Lewis-Benedict method

Hour	Blood Sugar Per Cent	Urine Volume	Urine Sugar
8.25 A.M.	0.096		
8.30	100 grams of glucose in 300 c.c. of water		
8.42	0.095	44	0
9.07	0.095	374	0
9.23	0.104	572	0
9.40	0.114	60	0
10.15	0.124	157	0
10.45	0.108	364	0
12.00	0.086	251	0

H. G. Weight 53 kg. Folin method for sugar determination †

Hour	Blood Sugar Per Cent	Urine Sugar
9.35	0.096	0
9.40	93 grams of glucose ingested	
10.40	0.130	0
11.40	0.142	0
12.30	0.101	0

* Hamman and Hirschman.
† Montefiore Hospital Records.

Endocrine and Nerve Control of Glycogenesis, Glycogenolysis and Glucolysis

Influence of the sympathetic nervous system and of the adrenals.

We now come to one of the most fascinating chapters in modern physiology. Claude Bernard, in the middle of last century, found that by puncturing the medulla, between the levels of origin of the vagus and auditory nerves of animals, he was able to bring about glucosuria, which was proven later to be the result of hyperglucemia. The intensity of the reaction was found to be directly related to the nutritional condition of the animal. Those that were well fed and contained a large amount of glycogen in the liver reacted very strongly, showing hyperglucemia and marked glucosuria; those that were starved and contained little glycogen in the liver reacted only feebly.

In 1901 Blum made the very important discovery that the injection of adrenalin was also followed by glucosuria, which was later proven to be the result of hyperglucemia. The adrenalin glucosuria resembled the puncture or piqure glucosuria, as it is called, in many respects. Its intensity is also dependent upon the amount of glycogen in the liver, and it also fails to produce hyperglucemia and glucosuria if the liver and muscles are free from glycogen.

It was further shown that repeated injections of adrenalin into animals with large amounts of glycogen will ultimately result in a complete discharge of all the glycogen from the liver.

A more intimate view of the relationship of the above two fundamental discoveries, one may gather from an analysis of the work carried out in Macleod's laboratory. First it was shown that by giving a sufficient amount of nicotine to cause a complete blocking of the sympathetic ganglia, the subsequent performance of the piqure experiment is followed by no glucosuria, indicating that the sympathetic nerve fibers may be the carriers of the impulses to the liver.

Secondly it was shown that by electrical stimulation of the great splanchnic nerve on the left side a very marked hyperglucemia may be produced.

It was further shown by G. N. Stewart that stimulation of the great splanchnic nerve is followed by the appearance of marked and easily detectable quantities of adrenalin in the blood of the suprarenal veins.

Lastly, it was shown by Mayer that after adrenalectomy in rabbits, piqure produced no hyperglucemia nor glucosuria.

From all the above, a chain of evidence seems to be established that piqure and adrenalin glucosuria are in reality one and the same kind of stimulation to the liver, and as we shall see later, every gland of internal secretion that possesses the power of sympathetic stimulation possesses

TABLE VIII

INFLUENCE OF ADRENALIN ON BLOOD SUGAR *

	Rabbit I		Rabbit II	
	Before Injection			
	Blood Sugar Per Cent	Urinary Sugar Per Cent	Blood Sugar Per Cent	Urinary Sugar Per Cent
	0.11	0	0.12	0
	After injection of 1.0 mg. of adrenalin subcutaneously			
15 minutes	0.18		0.16	
30 "	0.25		0.19	0.09
60 "	0.35		0.28	0.21
1½ hours	0.37		0.38	1.21
2 "	0.33	0.43	0.39	
2½ "	0.35		0.34	1.69
3 "				
4 "	0.24	1.55		
4½ "			0.27	3.55
5 "				
5½ "	0.16			
6 "				
6½ "	0.13	3.9		
7 "				
7½ "			0.12	3.11

* Bang's experiment.

the power, through its hyperactivity, to cause a discharge of the glycogen in the liver which is followed by hyperglucemia and glucosuria.

There is no interference with the animal's power to utilize carbohydrates, i. e., to oxidize it, after adrenalin administration.

Influence of the Pancreas.—In 1889 von Mering and Minkowski made the pathfinding discovery that the complete removal of the pancreas of an animal is followed by the appearance of marked glucosuria, with all the other symptoms of human diabetes. It was later found that with this glucosuria there runs parallel a very marked hyperglucemia. The glucosuria persists even if no carbohydrate is given in the food, and it was found that the sugar in the urine bears a definite relationship to the nitrogen that is excreted. For every gram of nitrogen that was found in the urine 2.8 grams of glucose were present. Since one gram of nitrogen is contained in 6.25 grams of protein, it is evident that the depancreatized dog has the power of converting 6.25 grams of protein into 2.8 grams of glucose.

The glycogen completely disappeared from the liver in spite of the high blood sugar concentration, and if carbohydrate was administered to the animal, it was quantitatively eliminated in the urine.

Experiments in which only portions of the pancreas were removed revealed that animals have a large "factor of safety" in their pancreas and

that by far the greatest portion can be removed with impunity. Of course there is a certain degree of variation in different animals, but in the great majority as much as four-fifths of the organ may be removed without producing any diabetes. When only very small portions of the pancreas are left intact, the animals develop a tendency towards alimentary glucosuria, but no true diabetes. The transition from this stage to that of true diabetes is entirely dependent upon the amount of pancreatic tissue left intact.

The most convincing proof that the absence of the pancreas was responsible for the glucosuria was presented by Minkowski in experiments in which he showed that animals that had their pancreas entirely removed did not develop diabetes if a portion of the pancreas was transplanted subcutaneously.

Since this was established attempts have been repeatedly made to extract a hormone from the pancreas and supply that to the depancreatized animals with the hope that the pancreatic function would be replaced. All attempts have failed, and the reason for it may be found in the fact that the digestive ferments of the pancreas destroy that hormone.

Two very interesting series of experiments were performed by Forschbach (1908 and 1913) and by A. J. Carlson and F. M. Drennan (1911).

Forschbach performed an operation on two dogs in such a way that the blood of dog A was made to circulate in dog B. He then completely removed the pancreas of dog B. As long as dog B received the blood from dog A, dog B did not develop any glucosuria, proving conclusively that the blood of dog A carries a substance (hormone) which takes the place of the pancreatic function. This was later corroborated by Hedon (1909), who found that the glucosuria of depancreatized dogs disappeared soon after he transfused it with the blood of a normal dog.

Carlson's experiments were based upon principles similar to the above, namely, that the blood carries a substance that is supplied to it by the pancreas. He therefore performed complete pancreatectomy in animals that were in the latter stages of pregnancy. Either very slight or no glucosuria set in. After the birth of the puppies, however, the mother became diabetic, proving that the fetus was able to supply the mother with its pancreatic hormone; true diabetes setting in after the fetal supply was removed.

There is therefore no more question to-day but that the pancreas is directly concerned with carbohydrate metabolism. It enables the body to oxidize glucose and it enables the body to convert glucose into glycogen. In its absence, or in case of its failure to functionate properly, the two functions disappear and the body loses the power to oxidize glucose and it also loses the power to convert glucose into glycogen, both of which result in hyperglucemia and glucosuria.

We are now confronted by the problem of how the pancreas exerts its influence on the carbohydrate metabolism. It will be a conservative esti-

mate to state that at least 200 publications have appeared on this subject.[9] Every conceivable theoretical possibility finds its defense and experimental support in one place and is met by just as convincing objection in another place.

That we are dealing with an internal secretion there is absolutely no question. That it is the pancreas that is supplying that internal secretion seems proved beyond doubt but its modus operandi and locus nascendi is as problematical to-day as heretofore. To the Islands of Langerhans we are now inclined to attribute the production of the "antidiabetic" hormones, but there is still room for direct and crucial experiments to prove this hypothesis.

Influence of the Thyroid Gands.—The thyroid influences the carbohydrate metabolism to a very considerable extent. Because it seems to have a stimulating effect on the entire plane of metabolism it undoubtedly affects the velocity of carbohydrate oxidation at the same time. Specifically it affects the carbohydrate metabolism in such a way that whenever there is a hyperfunction there is a tendency to lowered carbohydrate tolerance, i. e., hyperglucemia and glucosuria after the ingestion of 100 grams of glucose, and when there is a hypofunction, as in the case of cretinism and myxedema, we usually find a normal or increased tolerance for carbohydrates. (Janney and Isaacson, 1918.)

A great deal of confusion exists in the literature on the subject, probably because of the studies published on clinical cases that are not clearly defined. Because of the present tendency to attribute a great many cases of nervous disturbances to hyperthyroidism, one will naturally get a good many negative results. But when one examines the records of authentic cases of hyperthyroidism, one seldom fails to find evidences of a very marked lowering of the carbohydrate tolerance. Of interest in this connection is the observation of Jones (1893) and of Fr. Müller (1906(*c*)), both of whom reported the development of glucosuria in patients who were taking thyroid gland in excessive amounts. Von Notthaft (1898) also reports a case of true exophthalmic goiter complicated by glucosuria developing in an obese individual who had taken 1000 thyroid tablets in the course of five weeks.

There is no interference with carbohydrate oxidation in case of hyperthyroidism. The respiratory quotient after the ingestion of 100 grams of glucose, in the observations of DuBois (1916(*b*)), was 0.94 and 0.98, in the latter case showing that 89 per cent of the calories was derived from the glucose oxidation. On the other hand, the basal metabolism of the patient 17 hours after the last meal shows a respiratory quotient of 0.77,

[9] Excellent reviews of the literature up to 1908 are given by S. Rosenberg: "Innere Sekretion, Pankreas und Glykolyse," in Oppenheimer's Handbuch der Biochemie des Menschen und der Tiere. Vol. III, part I, pp. 245-270. And up to 1913 by F. M. Allen in Studies concerning Glycosuria and Diabetes, chapter XXI, pp. 898-985.

which indicates a low carbohydrate combustion which can only be explained on the basis of low glycogen reservoir. This is in conformity with the findings of Cramer and Kraus (1913) who found that after thyroid ingestion the liver does not retain glycogen as well as before.

The effect of the thyroid on carbohydrate metabolism, therefore, is purely through its interference with glycogen formation and mobilization. Its effect is similar to that of adrenalin and sympathetic stimulation, and the probabilities are, that they all act through the same channel.

Influence of the Pituitary Gland.—The pituitary gland, similar to the thyroid, has a tendency to affect the carbohydrate metabolism when in a state of hyperactivity. Cushing (1913) found that the administration of extract of the posterior lobe of pituitary was followed by a reduction in the carbohydrate tolerance and by a mobilization of glycogen. On the other hand, patients with acromegaly, who are supposed to suffer from an hyperfunctioning of the anterior lobe of the pituitary, very frequently show evidences of lowered carbohydrate tolerance and of glucosuria.

Borchhardt (1908) found glucosuria in 40 per cent of his cases of acromegaly, but in no case of tumor of the pituitary that was not acromegalic.

There is at present no reason to believe that the pituitary extracts affect the carbohydrate metabolism in any other way than do the extracts of the adrenals and thyroid. All three seem to have the power of stimulating the sympathetic nervous system, and the reaction they produce differs only in degree. The effect of adrenalin is most powerful; those of the thyroid and pituitary will only be determined after their respective effects have been studied with their active principles.

Just as the patellar reflex may be used clinically for roughly determining the state of nervous tension of an individual, so the carbohydrate tolerance test may be used clinically for determining roughly the state of an individual's tonus of the sympathetic nervous system. But we cannot employ that at present to differentiate between affections of the thyroid, pituitary or adrenal.

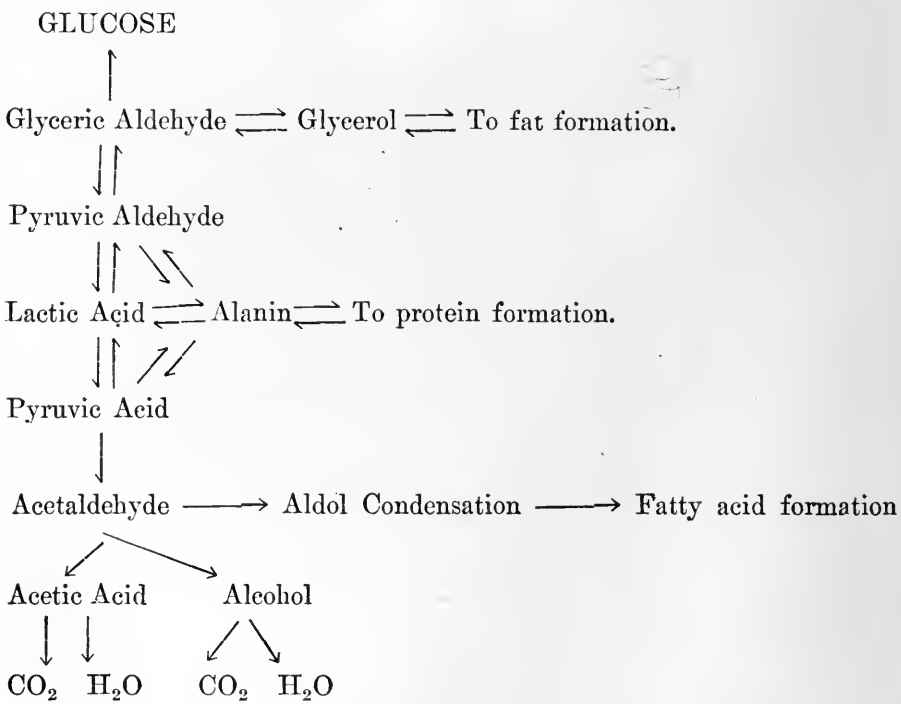
The Intermediary Metabolism of Carbohydrates

All the processes of metabolism aim at two objects, first to build up and maintain the body structure, second to produce the material that can be used for dynamogenetic purposes. It is most surprising that in spite of the large number of chemical compounds that play a rôle in metabolism, only very few are "fit to burn." In the chapter on protein metabolism it was brought out that fully fifty-eight per cent of the protein molecule passes through a glucose stage. Over ten per cent of the fat molecule (the glycerol fraction) passes through a glucose stage, and all of the

carbohydrates are converted into glucose. We can therefore see that glucose is the main channel of chemical action in the animal body, for from all sides the reaction swings in its direction.

But the cells of the body cannot oxidize glucose directly. The glucose molecule must first undergo a series of reactions during which it is broken up into much smaller and simpler compounds, and only those can be oxidized by the cells to yield energy. We may liken the process to the grinding down of grain to a flour in a mill, which is at the same time forcing the product through a series of sieves, each consecutive sieve having smaller and smaller meshes. Only those particles that can go through the finest mesh will be fit for consumption. All the others must be reground. One difference between the mill and the animal body is that in the mill the process is irreversible, that is to say, a particle that is once ground down remains so, whereas in the animal body the process is a reversible one, the particles possessing the power of again polymerizing and flying back into an upper sieve. The result is a continuous and endless grinding pressure from above and a continuous flying back to the upper sieves.

The grinding down process may be illustrated thus (the double arrow showing where the process is reversible).



The study of the intermediary metabolism of carbohydrates is fraught with great difficulty. In the first place we deal with substances that are

exceedingly soluble and therefore offer great technical difficulties in their isolation, purification and identification. Secondly, most of the substances are oxidized with great ease so that at no time can one find more than traces of them, even though throughout the twenty-four hours large quantities may have been produced. Our information therefore must be pieced together from various and indirect sources.

It has long been known that in the presence of alkali, glucose undergoes decomposition, giving rise to lactic acid. In the animal body lactic acid appears in the blood and urine in cases of asphyxiation, severe anemias, and after great muscular exertion. The following experimental proof shows that this lactic acid can have its origin in glucose. Mandel and Lusk (1906) found that after giving phosphorus to a dog lactic acid appeared in the urine in large quantities. When they administered phlorhizin to the same dog the animal of course became diabetic, and the lactic acid disappeared from the urine, indicating that the lactic acid could have been derived only from the catabolized glucose. This work is corroborated by von Fürth (1914, b) who found that the amount of lactic acid excreted in phosphorus poisoning is increased after administering glucose to the animal. Final and most convincing evidence was brought forward by Levene and Meyer (1913, b) when they showed that leucocytes and kidney tissues possess the power of converting glucose into lactic acid, and by Embden and Krauss (1912) who found that the addition of glucose to blood that is perfused through a surviving liver causes the appearance of considerable amounts of lactic acid.

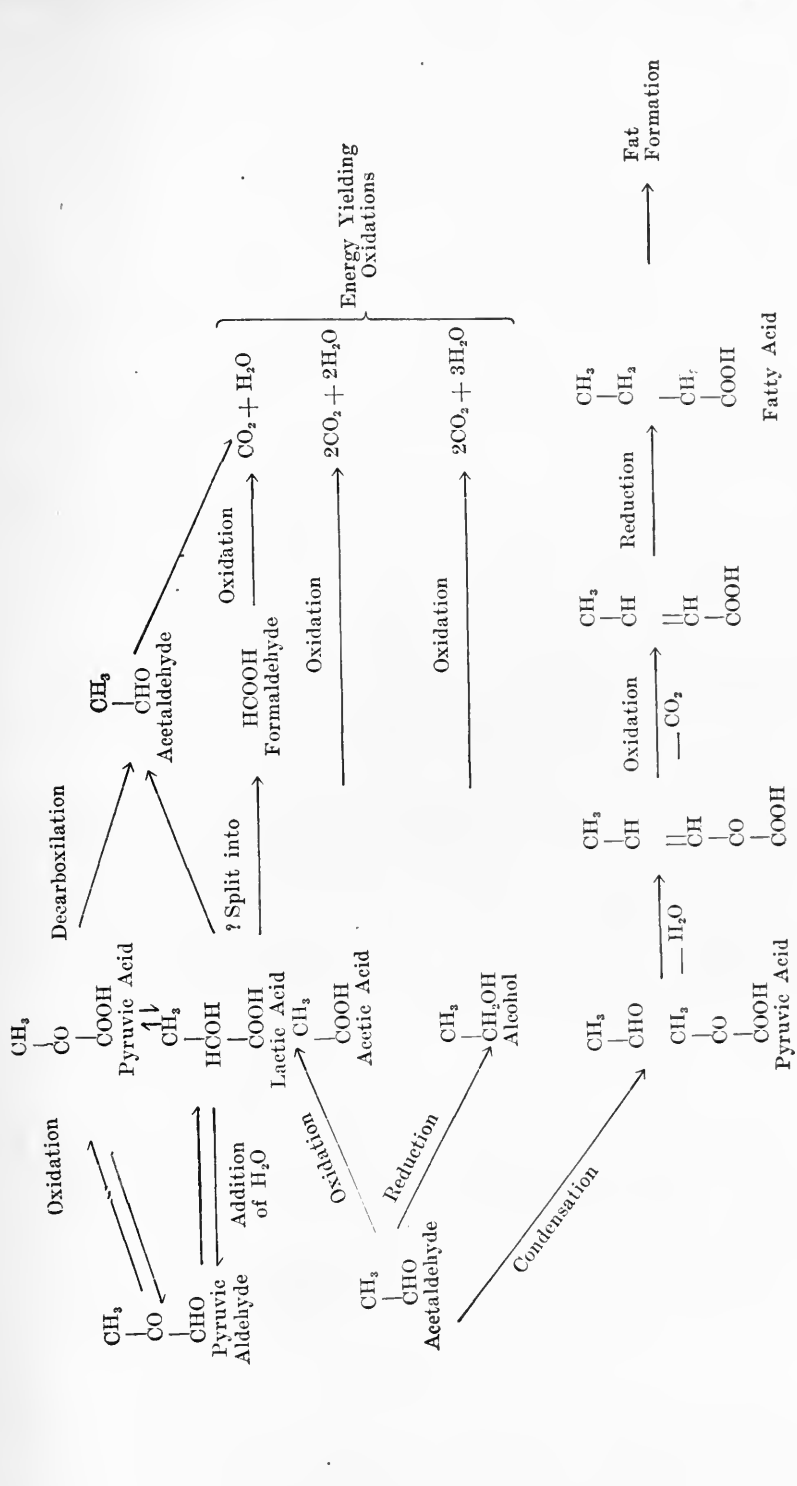
Embden, Baldes and Schmitz (1912) also demonstrated that washed blood corpuscles have the power of converting glyceric aldehyde into lactic acid to the same extent that they do glucose, indicating the possibility of glyceric aldehyde being an intermediary stage. They also showed that glyceric aldehyde when perfused through the liver is reduced to glycerol, and S. Oppenheimer (1912) added the information that glycerol when perfused through the liver gives rise to lactic acid.

Then follow experiments by Mayer (1912) in which he showed that after administering pyruvic acid to animals lactic acid appeared in the urine, and by Embden and Oppenheimer who obtained large amounts of lactic acid after perfusing the liver with pyruvic acid.

Finally, there is a whole array of experimental proof, showing with what ease various substances which are believed to be products of intermediary metabolism are converted back into glucose when fed to diabetic animals; for glyceric aldehyde, Woodyatt (1915); for dioxyacetone, Ringer and Frankel (1914(*c*)); for pyruvic aldehyde, Dakin and Dudley (1913); for pyruvic acid, Ringer (1913), Dakin and Janney (1913), Cremer (1913); for lactic acid, Mandel and Lusk (1906).

In the following chart the various reactions that may take place in the intermediary metabolism of glucose are indicated.

Chart IX
CH2OH
HCOH
HCOH
HOCH
HCOH
CHO
Glucose
Split into
CH2OH
HCOH
COH
CH2OH
HCOH
CHO
Glyceric Aldehyde
Rearrangement of Molecule
CH2OH
CO
CH2OH
Dioxyacetone
Splitting off of H2O
Splitting off of H2O
CH3
CO
CHO
Pyruvic Aldehyde


Oxidation
Decarboxilation
Pyruvic Aldehyde
Addition of H_2O
Pyruvic Acid
Acetaldehyde
? Split into
HCOOH
Formaldehyde
Oxidation
$CO_2 + H_2O$
Oxidation
$2CO_2 + 2H_2O$
Oxidation
$2CO_2 + 3H_2O$
Energy Yielding Oxidations
Lactic Acid
Acetic Acid
Oxidation
Reduction
Alcohol
Acetaldehyde
Condensation
$-H_2O$
Oxidation
$-CO_2$
Reduction
Fat Formation
Pyruvic Acid
Fatty Acid

We must picture these changes more from the dynamic point of view than from the static. We must realize that in every cell of the body the protoplasm is in constant motion. It is a system where hundreds of chemical reactions are going on continuously and almost simultaneously, where molecules are flying hither and thither, some undergoing oxidation, others undergoing reduction, and the whole struggling to reach an equilibrium. This struggle for chemical equilibrium constitutes the life of the cell. It is important also to bear in mind that the substances formulated in the chart do not normally represent *products* of intermediary metabolism, but rather stages or stations along a certain route of decomposition. The reaction does not stop at any of these points for any length of time to allow an accumulation of the products, except under abnormal conditions. For example. when the supply of oxygen is insufficient the process may halt at the lactic acid stage, then lactic acid can be detected in quantity. Just as an express train operating between New York and Chicago cannot arrive at its destination suddenly, but must go through certain stations along the route, so glucose must pass certain intermediary stages before reaching carbon dioxid and water. If the power does not hold out, naturally there will be a forced stop at one of the stations.

When we view the reactions on the chart we must also realize that there are two forces operative, one which drives the reaction downward and another which drives it backward to glucose. We are inclined to attribute them to the action of ferments. But ferments are blind forces that do not determine the direction of the reaction. Whether it goes to one side or another is controlled by physical chemical factors such as the mass action or relative concentration of the components. When the glucose concentration is high, the reaction swings in two directions with relatively great force and speed. Glucose is rapidly converted to glycogen on the one hand and to glyceric aldehyde on the other.

$$\text{Glycogen} \rightleftharpoons \text{GLUCOSE} \rightleftharpoons \text{Glyceric aldehyde}$$

But the reactions from glycogen to glucose and from glyceric aldehyde to glucose cannot be considered stopped. They probably go on at the same time, but the former reactions overshadow the latter. Similarly if glyceric aldehyde is fed to an animal we may picture the reaction in both directions, but going primarily in the line of least resistance.

$$\text{Glucose} \rightleftharpoons \text{GLYCERIC ALDEHYDE} \begin{matrix} \nearrow \text{Dioxyacetone} \\ \searrow \text{Pyruvic aldehyde} \end{matrix}$$

And so on with the other reactions.

On the basis of these last considerations one may find an explanation for the formation of glucose from practically all the intermediary metabolites of glucose when administered to diabetic animals. When one gives any of these substances to a normal animal the reaction of that substance

swings to left and right, that is, to glucose and downward. The particles that go over to glucose are ultimately broken down again, so that in the course of time the whole amount given is completely oxidized to carbon dioxid and water. Because of the relatively high concentration in the blood of the substance under discussion, the kidney may excrete some of it and also those products which stand nearest to it (excretion of lactic acid in the urine after pyruvic acid administration). But if the same metabolite is fed to a diabetic animal, the moment a particle is converted to glucose it becomes trapped, because these animals have lost the power of splitting the glucose molecule. The reaction becomes one-sided and irreversible, and if the oxidative processes are not very great the substance may be completely converted to glucose.

$$\text{Glucose} \longleftarrow \text{METABOLITE} \rightleftharpoons \text{Lower product}$$

It will now be readily seen that a number of three carbon compounds, namely glyceric aldehyde, dioxyacetone, pyruvic aldehyde, lactic acid and pyruvic acid, may be safely considered stages of glucose catabolism, and that these substances in the animal body may undergo reactions whereby one is converted into the others either by processes of oxidation, reduction, hydration, dehydration or by rearrangement of the position of hydrogen in the molecule. All of these steps are reversible.

One of the later stages in the reaction is a process of decarboxilation during which a three carbon compound is converted into a two carbon compound with the loss of carbon dioxid. This is the first irreversible reaction in the entire chain.

$$\begin{array}{l} CH_3 \\ | \\ CO \\ | \\ COOH \end{array} \longrightarrow \begin{array}{l} CH_3 \\ | \\ CHO + CO_2 \end{array}$$

Pyruvic Acid $\longrightarrow$ Acetaldehyde

That pyruvic acid can be converted into acetaldehyde was demonstrated in a series of experiments by Neuberg and Karczaz (1911, 1912). They found that all yeast cells possess that power and that the decarboxilation is brought about by an enzyme, "carboxylase."

Acetaldehyde is a very important intermediary stage of carbohydrate catabolism. Just as lactic and pyruvic acids link the carbohydrate metabolism with that of protein, so acetaldehyde links carbohydrate with fat metabolism. As will be shown later acetaldehyde is in all probability the starting point from which fat is built up in the body. Acetaldehyde in the organism may undergo oxidation to acetic acid which on further oxidation is converted to carbon dioxid and water. It may also be reduced to ethyl alcohol, which is ultimately oxidized to carbon dioxid and water.

It is only from these final oxidations that the cells of the body derive their energy. All the changes that the foodstuffs undergo, be it in the process of digestion or later in metabolism, are all aimed to prepare them for the stage in which the cells can utilize them for energy formation. Whether we start with the complex protein molecule, the high carbohydrate molecule or the comparatively simple fat molecule,—they must all be ground down in the mill of metabolism to fit the finest meshes of the sieve. They all have to come down to the two carbon stage which is burned with the liberation of heat and energy.

Fat Formation from Carbohydrate

That animals can be fattened by feeding them large amounts of carbohydrates has been known to stockmen for centuries. Scientific proof for it has been presented during the course of the last century by a number of authors.[10]

The question that confronts us to-day is, how can we picture the transfer of the highly oxidized glucose molecule to the oxygen poor fatty acid? It is chemically inconceivable that there is a direct abstraction of oxygen and that three glucose molecules become converted into an eighteen carbon fatty acid. We must therefore assume that the fatty acids are built up from more elementary compounds.

When one makes a survey of all the fats known in the animal and plant kingdoms, one is struck by the fact that in no place is there a natural fatty acid to be found that has an odd number of carbons. In milk, for example, there is present a variety of fatty acids. There we find,

Butyric Acid, $CH_3CH_2CH_2COOH$ (4 Carbons)

Caproic Acid, $CH_3CH_2CH_2CH_2CH_2COOH$ (6 Carbons)

Caprylic Acid, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2COOH$ (8 Carbons)

Capric Acid, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ (10 Carbons)

Lauric Acid, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ (12 Carbons)

Myristic Acid, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ (14 Carbons)

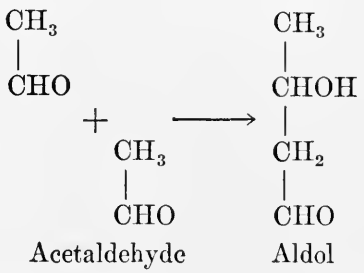
Palmitic Acid, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ (16 Carbons)

Stearic Acid, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$ (18 Carbons)

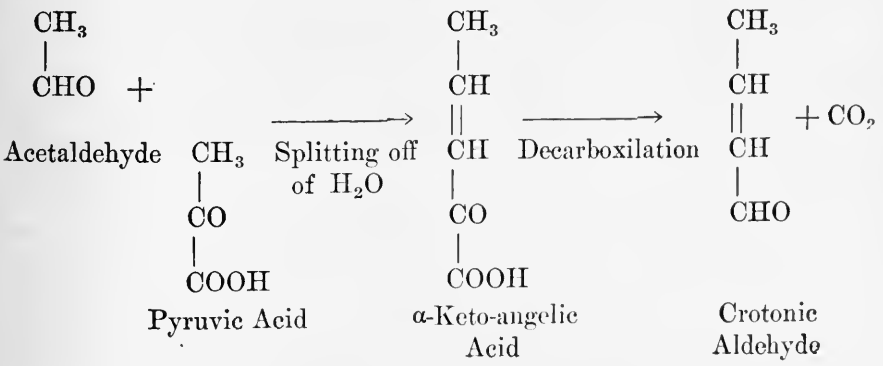
[10] A review of the literature may be found in "Die Fette im Stoffwechsel," by A. Magnus Levy and L. F. Meyer, in Oppenheimer's Handbuch der Biochemie des Menschen und der Tiere, vol. 4, part 1, p. 449, 1908.

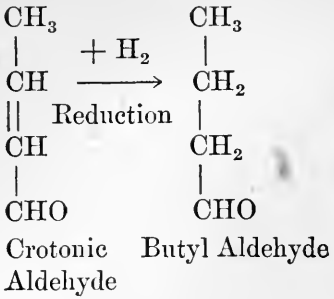
We have every reason to assume that all the lower fatty acids found in milk are intermediary in the building up of the higher fatty acids. If fatty acids were built up by the addition of one carbon we should find just as many odd carbon fatty acids as even. This consideration led Nencki as far back as 1878 to suggest that fatty acids are built up by consecutive additions of two carbons, and that the two carbon compound is probably acetaldehyde which displays exceptional chemical reactivity.

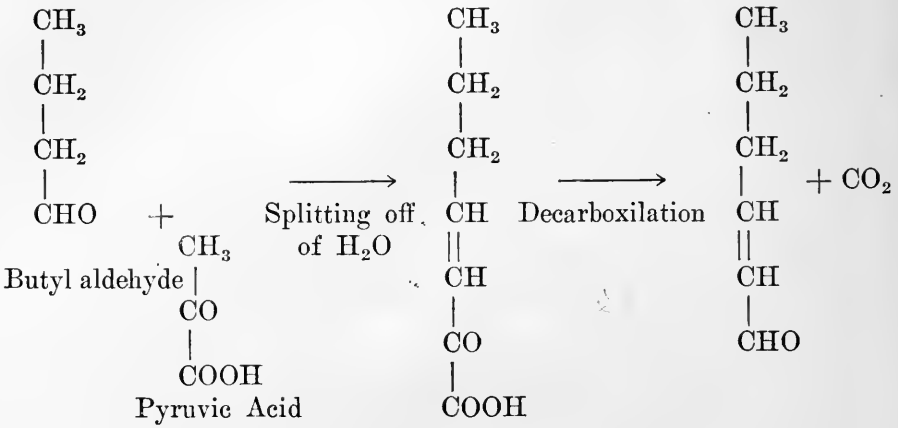
Support for this assumption may be found in the fact that in their catabolism fatty acids undergo a series of β-oxidation, whereby they lose two carbons in successive stages (Knoop (1910, *b*), Ringer (1913, *a*). *In vitro,* acetaldehyde will under certain conditions undergo what is known as aldol condensation, whereby one acetaldehyde molecule combines with another, forming aldol, which is a four carbon aldehyde. Raper (1907) has succeeded in building up an eight carbon aldehyde in this way, which he also easily oxidized to caprylic acid.

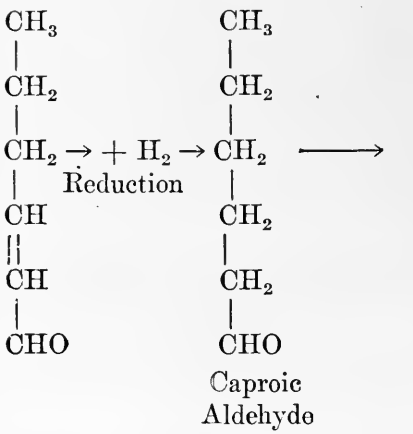


Smedley and Lubrynzka (1913) bring forth evidence that fat formation in the body proceeds through the condensation of an acetaldehyde molecule with that of pyruvic acid, forming first a four carbon aldehyde which later combines with another pyruvic acid molecule, giving rise to a six carbon aldehyde. The process thus repeats itself until the sixteen and eighteen carbon fatty acids are reached.









Unites with another molecule of pyruvic acid, and so on until the higher compounds are reached.

From the above we may see that fat formation can only take place in normal animals that have the power of splitting glucose, for the building stones, acetaldehyde and pyruvic acid, are mainly products of glucose catabolism In conditions of diabetes in which there is a loss in the individual's ability to break down the glucose molecule, fat formation from carbohydrate must be correspondingly reduced. This helps to account for the extreme and rapid emaciation in severe diabetes.

The Functions of Carbohydrate in the Diet.—The paramount function of carbohydrate in the diet is to yield energy to the cells in the process of its oxidation. It burns in the body apparently with greater ease than does protein or fat, hence it may be considered as having a sparing influence on both. With regard to protein its influence is more specific, for the intermediary products of carbohydrate metabolism, lactic acid and pyruvic acid have been shown to have the power of uniting with ammonia in the liver and giving rise to alanin. This conserves nitrogen for the body, which would ordinarily have been excreted, Knoop (1910), Embden (1910), and Schmitz (1910). For further discussion of the influence of carbohydrate on protein metabolism see the chapter on Protein Metabolism, page 118.

Influence of Carbohydrate on Intermediary Metabolism of Fat. Antiketogenesis.—Ordinarily when fat burns in the body it is completely oxidized to carbon dioxid and water. Under certain conditions, however, the oxidation is not complete. In cases of absolute starvation "acetone bodies" (β-hydroxybutyric acid, aceto-acetic acid, and acetone) appear in the urine, the last because of its extreme volatility is also excreted through the breath. If an individual is kept on a diet of protein and fat without any carbohydrate, these bodies will also appear in the urine. In severe diabetes where the combustion of carbohydrates is completely lost, the amount of acetone bodies formed may be enormous, over one hundred grams a day. Because the aceto-acetic acid and the acetone have the carbonyl (CO) radical, they are known as ketones and their formation in the body is called ketogenesis. All the acetone bodies originate from the catabolism of fat and from certain of the amino acids of protein metabolism.

Because it was recognized that whenever carbohydrates burn in the body ketogenesis stops and that no ketogenesis occurs as long as the body is capable of oxidizing glucose, antiketogenetic properties were attributed to glucose.

In normal fasting individuals who develop ketonuria, certain substances like glycerol, glycocoll, alanin, and aspartic acid have proven to be antiketogenetic. In diabetic individuals, however, they are without effect, because they are completely converted to glucose and excreted as such. Alcohol has proven to be a marked antiketogenetic substance. (O. Neubauer (1906), Benedict and Török (1906).)

In 1913 Ringer and Frankel performed a series of experiments on diabetic dogs who developed considerable ketonuria. After administering acetaldehyde to these dogs they found a very marked antiketogenetic effect. At the same time they also obtained an increase in the glucose elimination. They suggested the idea that it was possible that acetalde-

hyde acted by virtue of its combining power with β-hydroxybutyric acid, forming a new compound which is glucogenetic. We know to-day that acetaldehyde is a very important product in the intermediary metabolism of carbohydrate, and it is very likely that the antiketogenetic effect of glucose is brought about through acetaldehyde-β-hydroxybutyric acid or acetaldehyde-aceto-acetic acid combination.

Water as a Dietary Constituent . . . . . *Philip B. Hawk*

Introduction—Influence of an Increased Ingestion upon Metabolism—Influence on Basal Metabolism—Influence of a Diminished Water Intake—Water Drinking with Meals—Influence on Salivary Digestion—Influence on Gastric Digestion—Passage of Water from the Stomach—Influence of Pancreatic Digestion—Influence on Intestinal Flora and Putrefaction—Influence on Absorption—Influence on Blood Volume and Blood Pressure—Distilled Water—Ice Water—Conclusions.

Water as a Dietary Constituent

PHILIP B. HAWK

PHILADELPHIA

Introduction

The average man who lives among water mains, hydrants, and street sprinklers and in the vicinity of rivers and lakes gives little or no thought to the important part water plays in his life processes, if indeed he possesses any definite knowledge on the subject. If such a man were possessed of an introspective hydro-eye, he could quickly convince himself that "water" and "life" are synonymous terms so far as the human body is concerned. If he would flash the rays of this eye upon himself, he would find that the *blood plasma,* that important carrier of nutritive material to every organ and tissue, contains over 90 per cent of water; that the *brain,* which regulates and correlates so many intricate activities and processes, contains from 85 to 90 per cent water; that the *liver cell,* which is associated with so many processes which are vital to the maintenance of normal metabolism, contains 75 per cent water; that the mighty *muscle,* which is so importantly related to feats of strength, is three-fourths water; that the *saliva,* which quickly reduces the complex and insoluble starch of our foods to a simple soluble sugar, is almost pure water (99.5 per cent); that *bone,* which has been shown by test to possess a tensile strength (25,000 pounds per square inch) one and one-fourth times as great as that of cast iron and more than twice that of good timber, is 40 per cent water; and finally, if he would put his 150-pound body in an electric oven and drive off all the water, the undertaker would have to handle only 50 pounds, because the human body as a whole is about two-thirds water.

Since water is found in such large quantities in all organs, tissues, and secretions of the body, it is not surprising that water is absolutely essential to the proper performance of so many bodily functions. For example, in respiration we have chemical and physical processes which are dependent upon the presence of water. The surface of the lungs must be moist before there can be any exchange of carbon dioxid and oxygen. The regulation of body temperature is facilitated by the presence of circulating water and the evaporation of water from the surface of the

skin, whereas an increased water ingestion has been found to lower body temperature. The mucous surfaces of the body cannot function normally unless they are in a moist state. Water is the medium whereby nutritive material is carried to the body cells, and the cells of the blood are transported in a fluid medium. The kidney can more satisfactorily eliminate toxic substances if such substances are brought to that organ in a well-diluted form. The normal movement of joints and tendon sheaths is possible only when fluid is present. Water is also importantly related to absorption. The end-products of digestion in the intestine are not efficiently absorbed unless such end-products are properly diluted (see p. 291). Water also increases peristalsis. It has also been suggested (Smith and Mendel) that "The large amount of water in the cell may aid considerably in maintaining the optimum temperature of the cell, for water has a high specific heat. The large percentage of water in the tissues in which oxidation is most intense may be correlated with this unique property of acting as a heat buffer."

Inasmuch, therefore, as water is so vitally related to man's well being, it is not strange that water has been the object of considerable investigation by both the abstract scientist and the practical clinician.

That physicians, as long ago as the early part of the eighteenth century, were impressed with the dietary importance of water is indicated by a pamphlet published in London and reprinted in Philadelphia in 1723. This pamphlet is by John Smith, C. M., and is entitled "Curiosities of Common Water, or The Advantages thereof in Preventing and Curing Many Distempers." The author claims that the contents of the pamphlet were "Gathered from the Writings of several Eminent Physicians, and also from more than Forty Years' Experience." Among the interesting excerpts from the volume are the following:

"In the County of Cornwall, the poorer Sort, which did never, or but very seldom, drink any other drink but Water, were strong of Body, and lived to a very great age."

In another place the author of the volume quotes a Doctor Manwaring as saying:

"In the Primitive Ages of the World, Water-Drinkers were the longest Livers by some Hundreds of Years—nor so often sick and complaining as we are."

And later Sir Henry Blount is quoted as saying that while in the Levant "where the Use of Wine was forbid, and where the common drink was Water, he then had a better stomach for his Food, and digested it more kindly than he ever did before or since."

To-day practically all up-to-date medical men appreciate fully the importance of water to the human body. This fact is attested by the great development along certain hydrotherapeutic aspects of treatment. However, some doctors say to their patients, "Drink freely of water, at all

times *except during meals,"* and include almost invariably a warning against *ice water* and generally against *distilled water.* Such advice is analyzed in the following pages.

Influence of an Increased Water Ingestion upon Metabolism

That an increase in water intake will produce a change in the metabolic response of the human body has been repeatedly demonstrated (Eichhorst, Feder(*a*)(*b*), 1878, 1881, Falck, E. P. and F. A., Geuth, Gruzdiev, Matzkevich, Becher, Neumann(*a*), Panum, Rubner(*b*), Schöndorff(*a*), Weigelin, Hawk(*a*)). The consensus of opinion on this point is that an increase of 500-5000 c.c. in the daily water intake of a normal man will cause an increased excretion of total nitrogen, urea, phosphorus, and generally sulphur in the urine. The increase in total nitrogen and urea is believed to be due partly to the washing out of the tissues of the urea previously formed, but which has not been removed in the normal processes, and partly to a stimulation of protein catabolism. The increase in the excretion of phosphorus is probably due to increased cellular activity and the accompanying catabolism of nucleoproteins, lecithins, and other phosphorus-containing bodies. A typical nitrogen balance from one of the writer's experiments follows:

TABLE C.—INCOME AND OUTGO OF NITROGEN

EXPERIMENT I

Subject	Experimental Period	Length of Period Days	Nitrogen (grams)					Nature of the Diet
			In Food	In Urine	In Feces	Gain or Loss (+ or —)	Average Gain or Loss per Day	
I	I	2	25.68	22.13	2.95	+ 0.60	+ 0.30	Normal.
I	II	2	25.68	24.30	3.067	— 1.687	— 0.844	4500 c.c. water added daily.
I	III	4	51.36	44.82	4.568	+ 1.972	+ 0.493	Normal.
	Total	8	102.72	91.25	10.585	+ 0.885	+ 0.110	

In discussing the influence of water upon metabolism Bischoff, as early as 1853, wrote as follows:

"Water exercises before all other agencies, apart from the nitrogen content of the food, the greatest influence upon the excretion of urea by the urine."

And Foster, the eminent English physiologist, said in an early edition of his "Text-book of Physiology":

"Water has an effect on metabolism, as shown, among other things, by

the fact that when the water of a diet is increased the urea is increased to an extent beyond that which can be explained by the increase of fluid increasing the facilities of mere excretion."

The most direct evidence that an increased water ingestion increases cellular activity was furnished by an experiment made in the writer's laboratory (Howe, Mattill and Hawk (*a*), Wreath and Hawk).

A dog was given 700 c.c. of water daily during a 59-day fast, at which point the water ingestion was increased to 2,100 c.c. for each day of a four-day interval. The increased water intake caused an increased excretion of "total purin nitrogen," i.e., nitrogen in the form of purin bases, uric acid, and allantoin. Inasmuch as this form of nitrogen has its origin in the cell nucleus, we may consider that an increased output indicates stimulated cellular activity and increased tissue disintegration.

Certain other observations also indicate that water stimulates tissue changes. For example in the case of the fasting dog just mentioned, the increased water intake caused the appearance of considerable *creatin* in the urine. There had been no creatin in this dog's urine for a considerable interval before the high water intake. However, as soon as the water ingestion of the animal was increased, creatin appeared in considerable quantity in the urine. The creatin was interpreted as having arisen, at least in part, from disintegrated muscular tissue. The data on this point are embraced in the following table:

TABLE II

PERCENTAGE EXCRETION IN TERMS OF TOTAL NITROGEN

Day of Fast	Urea	Ammonia	Creatinin	Creatin	Purin	Allantoin	Undetermined
FASTING—700 C.C. WATER PER DAY							
54-57	85.57	9.31	5.76	....	0.50	0.37	....
58-59	85.28	8.55	5.75	....	0.57	0.42	....
FASTING—2100 C.C. WATER PER DAY							
60	79.54	9.20	4.38	0.67	0.10	0.71	5.41
61	80.76	9.81	4.71	1.92	0.11	0.65	2.03
62	78.49	12.63	4.59	1.03	0.06	1.16	2.04
63	78.88	10.17	4.94	1.61	0.07	1.00	3.33

Other observations made on men have been interpreted as indicating that a high water ingestion causes a partial muscular disintegration resulting in the release of creatin, but not profound enough to yield the total nitrogen content of the muscle. The output of creatin is, therefore, out of all proportion to the increase in the excretion of total nitrogen (Fowler and Hawk).

That the chloride content of the urine is increased as a result of an

augmented water intake has also been demonstrated (Heilner(*a*), Kast, Rulon and Hawk, Foster and Davis, Benedict(*a*)).

Influence on Basal Metabolism.—Apparently Speck is the only observer who has studied this question after the ingestion of volumes of water as great as those used in the writer's experiments, i.e., 3,000-4,500 c.c. per day. According to this observer, when 1,250 c.c. of water was taken, there was a noticeable rise in metabolism. Benedict and Carpenter (*b*) conclude that with more than 500 grams of cold water, an increase as great as 16 per cent above the basal value may be obtained.

Influence of a Diminished Water Intake.—If no water, or an insufficient quantity of water, enters our body, we quickly become abnormal. This point was emphasized in connection with a metabolism test in the writer's laboratory. We were to study the influence of an increased water ingestion. Therefore, in order to have a pronounced difference between the water intake of the preliminary and experimental periods, the water quota of the diet of the preliminary period was reduced to a minimum. The subjects (men) of the experiment soon gave evidence of abnormal function as shown by headaches, nervousness, loss of appetite, digestive disturbances, and inability to concentrate on the performance of accurate chemical work. As soon as the above symptoms appeared, the water content of the diet was increased, and with this single change the experiment proceeded satisfactorily. Dennig and Niles have also shown the undesirable effect of a diminished water intake.

That man or a lower animal *will live longer without food than without water* is well recognized. If we give a dog all the food he wishes but no water, the beast dies in a short time. If we give the animal no food but see to it that he receives plenty of water, the animal will live much longer. In a test in the writer's laboratory in 1912 (Howe, Mattill, and Hawk(*b*)), an adult dog (26 kg.), which was given 700 c.c. of water daily, lived over 100 days without food. Smirnov has also demonstrated that fasting rabbits which were permitted free access to water were less prone to show signs of fatty infiltration of the liver than were similar fasting rabbits which were not permitted to drink water.

Rubner says that a fasting animal may lose all its glycogen and fat and one-half its protein and still live, *but if it loses one-tenth of its water, it dies.* We are continually losing water by way of the kidneys, lungs, skin, and bowel, and if we do not drink sufficient water to make good these losses, our body quickly ceases to function properly and death soon follows. That the loss of water through skin and air passages may be considerable has been shown by direct determination (Soderstrom and DuBois). Normal men twenty to fifty years old may lose by these channels 700 grams of water per day, and the water thus lost carries with it 24 per cent of the total heat produced in the body. Typhoid patients with a rising temperature show a decreased water output, while the reverse is

true when the temperature falls. In general, however, the output of water is very little affected in disease.

That a lack of free water in the body may bring about a rapid and high increase in body temperature has been demonstrated (Balcar, Sansum, and Woodyatt, Woodyatt(*a*)). When sugar, for instance, is injected intravenously in a dog and the animal is given no water, high fever and chills soon follow. Temperatures as high as 120° F. have been obtained by this method. The sugar produces diuresis, causing a lack of water in the dog's body, and the fever and high temperature follow.

Certain well known pathological conditions are associated with a loss of water from the body. In fatal cases of Asiatic cholera, for example, this desiccation takes place to such an extent that we may have a serum loss as high as 62 per cent (Rogers). If isotonic saline be injected intravenously into such cholera patients, the fluid is immediately and completely lost by way of the bowel. In cases of poisoning by war gas (Underhill), there is also a pronounced loss of water from the blood and the movement of water into the lungs. The pneumonia crisis in infants (Lussky and Friedstein) has been shown to be accompanied by decrease in body weight due to loss of water.

Water Drinking with Meals.—Beginning in 1908, a long series of studies have been carried out in the writer's laboratory bearing upon the question of water drinking at meal time. At the time our first study was made, the consensus of medical opinion was opposed to the mid-meal use of water. Oertel, who was an advocate of fluid restriction, says, "The drinking of fluids with meals causes great dilution of the gastric juice, retards gastric digestion, and favors the development of dyspepsia." The following quotation (Carrington) will also serve to emphasize, in a general way, some of the reasons why physicians were opposed to the drinking of water with meals:

"We can lay down the definite and certain rule that it (water) should never be drunk at meals, and preferably not for at least one hour after the meal has been eaten. The effect of drinking water while eating is, first, to artificially moisten the food, thus hindering the normal and healthful flow of saliva and the other digestive juices; secondly, to dilute the various juices to an abnormal extent; and thirdly, to wash the food elements through the stomach and into the intestines before they have had time to become thoroughly liquefied and digested. The effect of this upon the welfare of the whole organism can only be described as direful."

However, if we search for experimental proof of the above statements, we fail to find it, no matter how deeply we dig into the musty volumes of scientific and medical libraries. In all my search I have never found a single experimental fact which can rightly be interpreted as indicating that the taking of water at meal time is harmful. In none of our tests was water used to wash down the products of incomplete mastication; the

food was invariably masticated without the aid of water. Let us follow the various activities of the digestive tract, from mouth to anus, and see the actual influence of water taken with meals upon these activities.

Influence on Salivary Digestion.—It is not necessary to believe with Bunge that the main function of the saliva is one of lubrication, in order to show that the presence of water aids salivary digestion. The following table (Bergeim and Hawk) shows that the dilution of saliva with water facilitates the action of the salivary amylase:

EFFECT OF DILUTION OF SALIVA IN CONCENTRATED MIXTURES

Diluent: Filtered tap water. Time of digestion: 10 min. Temp.: 0°.

No.	Amount of Starch Paste	No. cc. Saliva	Amount of Water	Mg. of Maltose	Dilution 1:
1.....	10 cc. of 10%	10	.....	378.6	2
2.....	7 cc. of 10%	7	6 cc.	441.8	3
3.....	4 cc. of 10%	4	12 cc.	448.6	5
4.....	**3 cc. of 10%**	**3**	**14 cc.**	**458.5**	**7**
5.....	2 cc. of 10%	2	16 cc.	449.3	10
6.....	1 cc. of 10%	1	18.0 cc.	305.4	20
7.....	0.4 cc. of 10%	0.4	19.2 cc.	283.0	50
8.....	0.2 cc. of 10%	0.2	19.6 cc.	287.6	100

The diluent in the above experiment was ordinary tap water, and the optimum dilution was *six volumes of water.*

Influence on Gastric Digestion.—(*Stimulatory Power of Water*).—The most severe indictment brought against the drinking of water with meals was the claim that water thus taken would *dilute the gastric juice* and hence delay digestion. Those who advanced this criticism overlooked the fact that the gastric juice is manufactured by living cells which are subject to chemical and psychical stimulation and that water is a chemical stimulant. The first experiments showing that water possessed the power to stimulate the flow of gastric juice were apparently made in 1879 (Heidenhain). This observation was later repeatedly confirmed by other investigators (Carlson, Orr, and Brinkman, Foster and Lambert, King and Hanford, Lönnquist, Pavlov, Sanotzky, Sawitsch and Zeliony), all of whom used lower animals as subjects. Pavlov was not impressed with the stimulatory power of water—in fact, he found no stimulation whatever in about 50 per cent of his tests where volumes of water ranging from 100 to 150 c.c. were introduced into the stomachs of dogs. He says:

"It is only a prolonged and widely spread contact of the water with the gastric mucous membrane, which gives a constant and positive result."

Foster and Lambert also claimed that volumes of water below 200 c.c. exerted no appreciable or uniform stimulation in the stomach of the dog. According to these investigators the increase in the flow of gastric juice

which follows the introduction of water is directly proportional to the volume of water employed. This point is shown in the following data taken from one of their tests:

300 c.c. water = 7.2 c.c. gastric juice
500 c.c. water = 17.7 c.c. gastric juice
750 c.c. water = 25.7 c.c. gastric juice

Chighin had previously shown a similar proportionality. The observations mentioned were made by the use of the Pavlov pouch.

The first experiments showing water to be a gastric stimulant in the human stomach were made in the writer's laboratory (Wills and Hawk). The ingestion of water at meal time by two men was accompanied by an increase in the excretion of ammonia which was directly proportional to the extra volume of water ingested. Inasmuch as certain experiments have demonstrated that water stimulates the flow of an acid gastric juice and as certain other experiments have demonstrated that the formation of acid in the body or the introduction of acid from without produces an increase in the urinary ammonia excretion, we feel justified in assuming that the increase in the ammonia excretion observed in our experiments was due directly to the stimulation of gastric secretion by the ingested water. That the increase in the ammonia excretion did not arise from intestinal putrefaction was indicated by the finding of lowered indican values during the period of high water ingestion. These observations were verified by Ivy(a) in experiments on dogs.

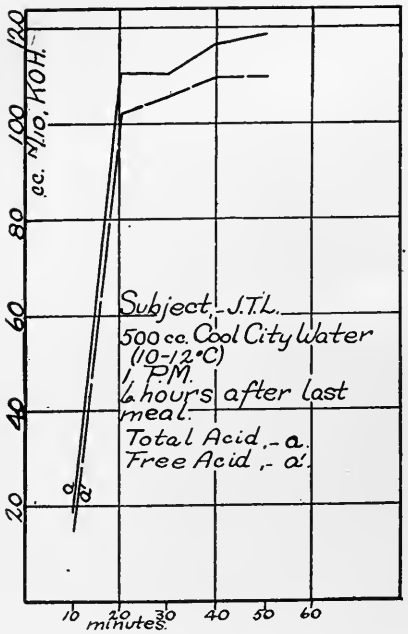


Fig. 1.—Curve showing pronounced stimulation by water and rapid emptying of the stomach. (Bergeim, Rehfuss and Hawk; Jour. Biol. Chem., 1914, XIX, 345.)

Since these observations gave only "indirect" data, the problem was reinvestigated in the writer's laboratory and "direct" evidence of stimulation obtained. In the latter investigation (Bergeim, Rehfuss and Hawk), water was introduced into the stomachs of normal men and samples of gastric contents removed at intervals of ten minutes by means of the Rehfuss tube (Rehfuss) and analyzed according to the fractional method of gastric analysis (Hawk (g)). Figure 1 illustrates a pronounced case of water stimulation of gastric secretion, and Figure 2 illustrates a stimula-

tion of moderate intensity, whereas Figure 3 shows but slight stimulation. These tests were made on three men who gave normal gastric histories, and serve to illustrate the fact that all normal stomachs do not yield the same response to chemical stimulation. This point has been emphasized throughout our work on "Gastric Response" (Miller, Fowler, Bergeim, Rehfuss, and Hawk). In other words, water is an important gastric stimulant, but it does not exert a pronounced stimulatory effect in every normal stomach—neither does any other dietary article. This same fact has also been brought out by Ivy(*b*). Other interesting water experiments have also been made by Sutherland, and by King and Hanford. The latter investigators say:

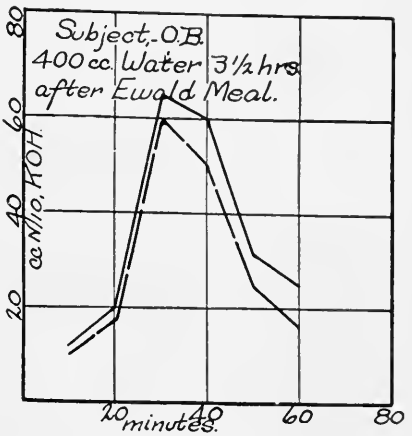


Fig. 2.—Curve showing moderate stimulation by water (Bergeim, Rehfuss and Hawk: Jour. Biol. Chem., 1914, XIX, 345.)

"Water given with meals or during digestion results in the following hour in an increase in the amount of juice secreted over that which would be secreted on the administration of either water or meat alone."

Niles, as the result of experiments on eight men, each of whom received one liter of water at each meal for one week, also approves of water drinking with meals. He says, "Not one of the eight suffered a single qualm of indigestion, either gastric or intestinal."

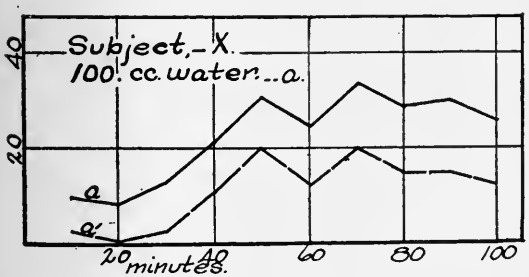


Fig. 3. Curve showing slight stimulation by water in the human stomach. (Fowler, Rehfuss and Hawk; unpublished data.)

That the water sometimes begins its stimulation as soon as it comes in contact with the human gastric mucosa is illustrated by Fig. 4. In this experiment, after removing the gastric residuum (Rehfuss, Bergeim and Hawk(*a*); Fowler, Rehfuss, and Hawk) of a normal man, 100 c.c. of water was introduced into the empty stomach through the Rehfuss tube. That there was no latent period is shown by the fact that an acidity of 15 was registered at the end of one minute, and this value had risen to 80 at the end of a five-minute interval. Pavlov claims that the stomach of the dog shows a latent period of five minutes, whereas

other observers (Bogen, Hornborg, Kaznelson, Sick, Umber) claim, as the result of experiments on man, that the latent period varies from 3 to 10 minutes. Carlson says:

"The latent period of the appetite secretion varies indirectly with the rate of continuous secretion so that when the continuous secretion is abundant, the appetite secretion shows no latent period at all, while with the lowest rate of the continuous secretion, the latent period varies from 2 to 4 minutes."

That this latent period does not exist in certain human stomachs after water stimulation is evident from our data.

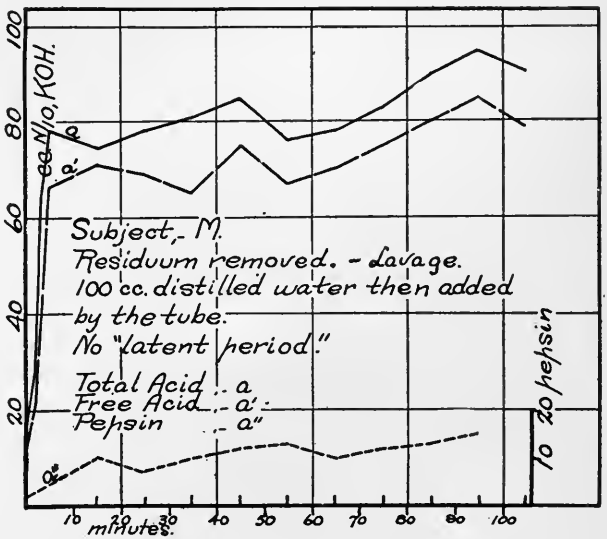


Fig. 4.—Curves showing immediate stimulation by water and rapid emptying of the stomach. (Bergeim, Rehfuss and Hawk; Jour. Biol. Chem., 1914, XIX, 345.)

It has also been claimed that the gastric glands exhibit a pronounced fatigue when subjected to repeated stimulation (Foster and Lambert). That this pronounced glandular fatigue is not always in evidence is illustrated in Fig. 5. A normal man was given 500 c.c. city water (10°-12° C.) at 1 p. m., five hours after breakfast, and samples of juice were collected at ten-minute intervals until the stomach was approximately empty. After an intermission of ten minutes the experiment was repeated. It will be observed that the stimulation was almost as great in the repeated test as in the initial one. A similar absence of glandular fatigue, in the dog, has also been observed by Ivy(*b*) after the injection of gastrin every two hours over a period of twenty-six hours.

When gastric stimulants are under discussion, much emphasis is invariably placed upon the stimulatory power of meat extract. The comparative stimulatory power of water and meat extract in the same normal

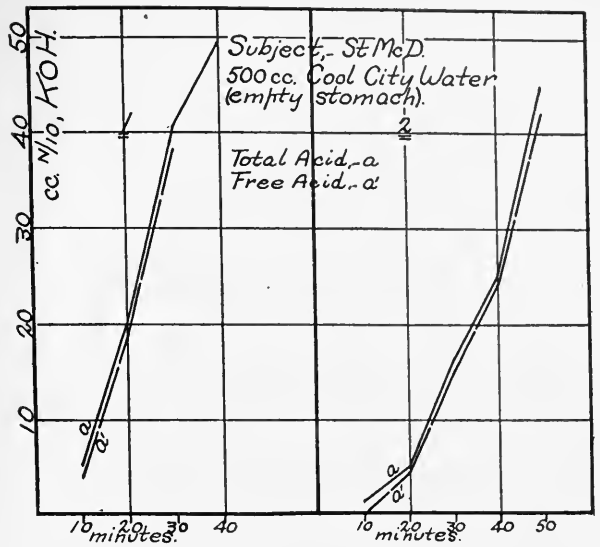


Fig. 5.—Curves showing no glandular fatigue in human stomach. (Bergeim, Rehfuss and Hawk; Jour. Biol. Chem., 1914, XIX, 345.)

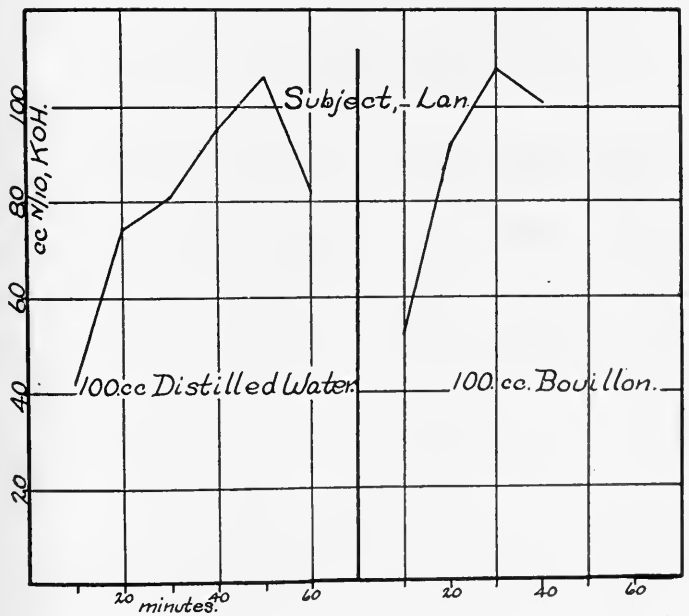


Fig. 6.—Curves showing comparative stimulatory power of water and bouillon in the human stomach. (Fowler, Rehfuss and Hawk; unpublished data.)

stomach is illustrated in Fig. 6. It will be observed that the gastric acidity was developed a little more quickly in the case of meat extract, and the stomach emptied a little more rapidly, but that the general stimulatory response was very similar to that of water. Fig. 7 shows the comparative stimulation produced by water and coffee. Here again it will be observed that the response is very similar in the two cases. The above protocols give emphasis to the belief that the stimulation produced in the stomach by aqueous solutions of various kinds is due many times in large part to the water alone.

That water may sometimes stimulate the stomach fully as much as certain common foods is illustrated in Fig. 8. Here we have a direct comparison with oatmeal, a good standard food, and it will be noted that water exerted a greater stimulation than the food in question.

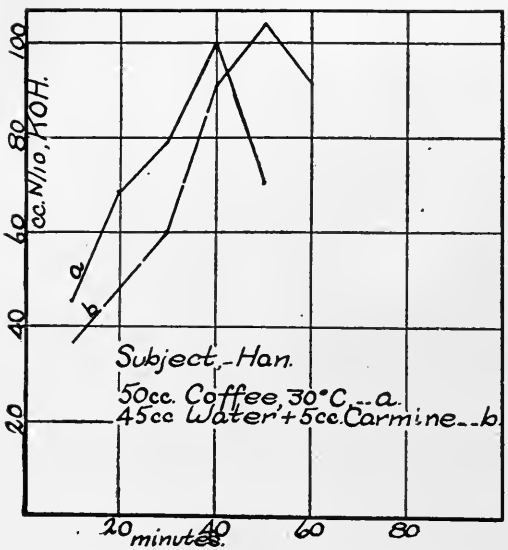


Fig. 7.—Curves showing comparative stimulatory power of water and coffee in the human stomach. (Fowler, Rehfuss and Hawk; unpublished data.)

That Pavlov's claim, based on animal tests, that water stimulates gastric secretion only when there is "widespread and prolonged" contact with the gastric mucosa, does not hold, for the human stomach has been demonstrated repeatedly in our work. Pronounced gastric stimulation with high acid values and rapid stomach evacuation have been obtained after the introduction of as small a volume as 25 to 50 c.c. of water into a normal human stomach.

Passage of Water from the Stomach.—If water remained in the stomach for long periods of time after its ingestion, there might be some argument against its free use with meals. However, there is abundant evidence that it leaves very rapidly (Cohnheim(*a*), Grützner(*a*)(*b*), 1902, 1905, Gröbbels, Kaufmann, Leconte, Scheunert, Gabrilowitch). Grützner says:

"Mässiges Getränk während der Mahlzeit stört sicherlich die Tätigkeit des gesunden Magens in keiner Weise, wie man vielfach angenommen hat."

Leconte, who fed two dogs normally, 2 hours later gave one of them water, and 15 minutes later examined the stomach contents of both animals. He found scarcely any difference between the two, the water

having largely left the stomach and even the duodenum. The general consensus of opinion is that water leaves the stomach rapidly, the bulk of it in the first few minutes along the so-called "Rinne," or trough, in the lesser curvature, this being particularly true of the empty stomach. Waldeyer and Kauffmann established the presence of this trough on anatomical grounds, Ernst contributed evidence from a pathological standpoint, and Cohnheim apparently succeeded in directly observing this phenomenon in his experiments on dogs. Scheunert, on the other hand, takes the opposite view and claims, from his experiments on the horse's

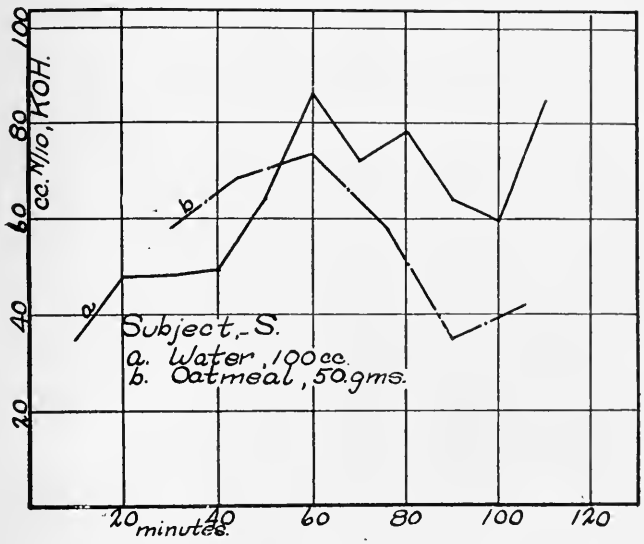


Fig. 8.—Curves showing comparative stimulatory power of water and oatmeal in the human stomach. (Fowler, Rehfuss and Hawk; unpublished data.)

stomach, that liquid in the distended stomach has a tendency to permeate along the gastric walls.

The effect of water combined with foodstuffs has also been the subject of interesting experiments. Gröbbels is authority for the statement that in dogs the digestion of bread followed by water is shorter than that of bread alone. Gabrilowitch demonstrated that in the administration of a mixture of meat and water the water passes out of the stomach, allowing the meat to follow its customary digestion. Certain experiments in the writer's laboratory also furnish evidence that water, at least in some cases, leaves the stomach very quickly. In this connection please refer to Fig. 1, p. 282. In this experiment, a normal man received 500 c.c. of water six hours after the last meal. Twenty minutes after the water passed into the stomach, the gastric contents showed an acid value of 111.5, and these figures were not subsequently materially altered. We believe that the data from this test furnish evidence of the rapidity with which

the water left the stomach. We may believe that the 500 c.c. of water upon reaching the stomach at once stimulated the gastric glands to greater activity, and caused the contents of the stomach to assume an acidity of 19.0. Some time during the next ten minutes, i.e., ten to twenty minutes after the water first reached the stomach, practically the entire 500 c.c. had passed into the intestine and left behind a gastric juice of high acid concentration (111.5). That the stomach was practically empty in from 10 to 20 minutes, as far as the original water was concerned, is indicated by the uniform values obtained for acidity in the samples withdrawn from the stomach during the next half hour. In other words, we believe that the only acidity value which was influenced by the factor of dilution was the acidity value of the ten minute sample. Some time before the next specimen was taken the large volume of water had passed into the intestine and our acidity value (111.5) represents the true stimulatory power of the water unmasked by the factor of dilution. This is an example of the hypersecretory type of stomach which we have discussed in our publications (Rehfuss, Bergeim and Hawk(*b*)).

Another illustration of a stomach which rapidly emptied after the entrance of water is given in Fig. 4. Here we have an acidity of 80 developed in five minutes after the entrance of 100 c.c. of water into an empty normal human stomach. Inasmuch as the acidity values did not materially change during the next hour and forty minutes we feel safe in interpreting the data as indicating a practically complete emptying of the stomach inside of ten minutes. That water and other dietary fluids, such as coffee and tea, do not delay the emptying time of the stomach, when taken with food, has also been shown in the writer's laboratory (Miller, Bergeim, Rehfuss, and Hawk). Four normal men were used as subjects. The evacuation time after a standard mixed meal had been eaten was first determined and in later tests the evacuation time of the same meal plus a liter of water, coffee, or tea was studied. The data are summarized in Fig. 9.

Summarizing the various experiments which have been made to learn the influence of water in the human stomach, we may conclude as follows: The introduction of water immediately stimulates the gastric glands to increased activity. In a few minutes, the bulk of the water so introduced leaves the stomach and does not interfere with the evacuation of that organ while its stimulatory action persists, causing the outpouring of a highly active gastric juice which insures efficient gastric digestion. *It is, therefore, better to drink water with meals than between meals.* If taken between meals, we have the same stimulatory effect on gastric secretion, but there is nothing in the stomach to digest, and we have thus a true economic waste. A summary of the experiments on water drinking with meals is contained in a publication by the writer (Hawk (*e*)).

Influence on Pancreatic Digestion.—Pavlov has shown that when 150 c.c. of water are introduced into the stomach of a dog, the pancreas begins to secrete, or augments its flow, within a few minutes after the water has entered the stomach. Since this investigator found 150 c.c. of water insufficient to excite a flow of gastric juice, the secretion of pancreatic juice is apparently not secondary to a secretion of the other, but is a direct result of the presence of water in the stomach. In the case of man, however, we have shown that water is a pronounced gastric stimulant and causes the passage of large quantities of acid chyme into the intestine. Inasmuch as this acid acts as a pancreatic stimulant, we have, therefore, an indirect

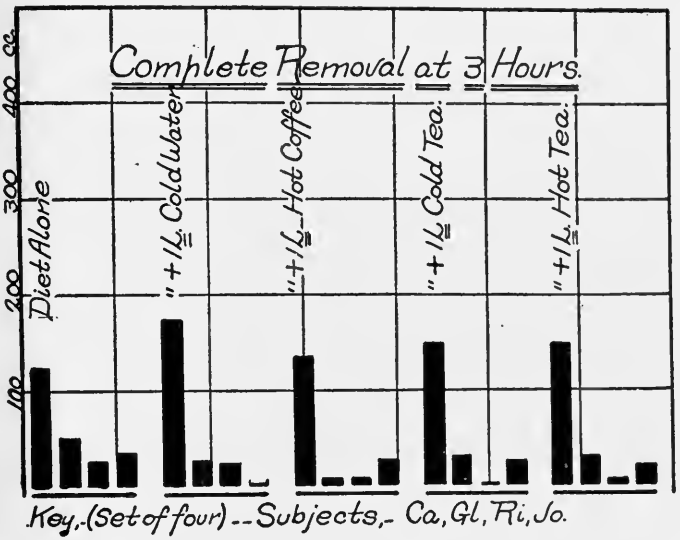


Fig. 9.—Chart illustrating the evacuation of various fluids from the human stomach. (Miller, Bergeim, Rehfuss and Hawk; Am. Jour. Physiol., 1920, LII, 28-53.)

stimulation of pancreatic secretion (Hawk(*d*), 1911). On the basis of the data gathered in the investigation just mentioned and in associated investigations made in the writer's laboratory and elsewhere, we are prepared to draw the general conclusion that the ingestion of quantities of water at mealtime ranging in volume from ½ to 1 1/3 liters stimulates the pancreatic function in two ways: first, a direct stimulation of the nervous mechanism of the pancreas brought about while the water is still in the stomach and, second, an indirect stimulation brought about on the entrance of the increased volume of acid chyme into the duodenum. If we have this augmented pancreatic activity, we would expect to find a more efficient pancreatic digestion when water is taken with meals. Certain of our experiments (Mattill and Hawk(*b*)) have demonstrated this point. The experiments in question were performed on men living on a uniform diet; a preliminary period of small water ingestion was followed by a

period of large water ingestion with meals, and this, in turn, by a final period with the original conditions. When one liter of water additional was taken with meals the average daily excretion of fat in the feces was much reduced below that found when a minimum amount of water was taken with meals; one and one-third liters had a like effect. A similar but less marked reduction was observed when 500 c.c. of water were taken with meals.

The decreased excretion of fat observed during water drinking with meals was usually evident for a number of days after water had ceased to be taken in large or moderate amounts with meals indicating that the beneficial influence of water was not temporary but was more or less permanent. After several months of moderate water drinking with meals a pronounced improvement in the digestibility of fat was observed, the percentage utilization having risen from 94.3 to 96.5. A slight gain in weight accompanied the water drinking, and this gain was not subsequently lost.

The better digestion and absorption of fat was probably due to the following factors:

(1) *Increased secretion of gastric juice and of pancreatic juice as a result of the stimulating action of water.*

(2) *Increased acidity of the chyme bringing about a more active secretion of pancreatic juice and bile.*

(3) *Increased peristalsis due to larger volume of material in the intestine.*

(4) *A more complete hydrolysis of the fats by lipase, due to increased dilution (Bradley(a)) of the medium and consequently more rapid absorption.*

Certain of our experiments on carbohydrate digestion are also of interest in this connection. It has been shown (Mattill and Hawk, 1911), for example, that in men living on a uniform diet the addition of 1,000 c.c. of water to each meal causes a decrease in excreted carbohydrate material. The better utilization of food material thus evident was not temporary but appeared to extend for some time following the use of water. The ingestion of a smaller amount of water (500 c.c.) and the use of a large volume of water (1,333 c.c.) by one accustomed to drinking water with meals showed a similar but less marked reduction in the excretion of carbohydrate.

Other experiments on protein digestion and absorption point in the same direction (Mattill and Hawk(*d*)). These studies showed that the drinking of three liters of water *with meals* caused a more economical utilization of the protein constituents of the diet. Gains in body weight were also registered.

Influence on Intestinal Flora and Putrefaction.—Since absorption is more rapid and complete when water is taken with meals, there will be less food material remaining in the intestine to furnish pabulum for intestinal organisms. We would, therefore, expect to find a diminished output of such organisms in the feces and a decreased intestinal putrefaction. These facts have been emphasized by certain of our experimental findings (Mattill and Hawk(*c*), Fowler and Hawk, Blatherwick and Hawk(*a*)). In one instance, the excretion of bacterial dry substance in the feces was reduced from 8.0 grams to 6.2 grams per day as the result of drinking about a liter of water per meal for a period of five days.

That intestinal putrefaction is reduced when water is drunk freely at meal time has also been shown using indican as the index (Sherwin and Hawk, Hattrem and Hawk). The decreased intestinal putrefaction brought about through the ingestion of moderate (500 c.c.) or copious (1,000 c.c.) quantities of water at meal time was probably due to diminution in the activity of indol-forming bacteria following the accelerated absorption of the products of protein digestion, and the passage of excessive amounts of strongly acid chyme into the intestine.

Influence on Absorption.—The better utilization of the fat, carbohydrate and protein of the diet as just discussed furnishes proof that the drinking of water facilitates the absorption of the products of the digestion of our food. The drinking of water dilutes the material in the intestine and aids in its absorption. Concentrated solutions are not readily absorbed, as is shown by the experiments of London and Polovzova(*a*) and others. The latter investigators showed that when concentrated solutions of glucose are introduced into the intestine, a diluting secretion begins to flow from the wall of the intestine. Its amount runs parallel with increasing concentration of the glucose solution, and at its maximum it may amount to one-half the total quantity of blood in the animal. By this dilution and also by absorption of sugar the concentration of the solution is brought down to 6-8 per cent, a dilution at which absorption takes place very readily in the lower intestinal tract. The secretion of the diluting fluid begins with the coming in of the first glucose solution and continues fairly uniformly. Since absorption is going on more or less continuously in the intestine, the water taken with one meal aids in diluting the products of the previous meal which are in the intestine. Not only is enzyme action more complete in dilute solutions but such solutions are also better adapted to absorption. When the solutions to be absorbed are not dilute, the organism must first make them so by pouring out a diluting secretion; if they have been made dilute, the organism is spared this task.

Influence on Blood Volume and Blood Pressure.—The practice of drinking large volumes of water is sometimes criticized on the theory that it increases blood volume and consequently causes a rise in blood pressure. However, some Yale experiments (Bogert, Underhill and Mendel)

have shown that there is complete restoration of blood volume of the dog and rabbit within thirty minutes after the intravenous injection of a quantity of saline equal to the calculated blood volume of the individual. Therefore, after one drinks copiously of water, the influence upon blood volume and blood pressure is both slight and transitory.

Distilled Water.—A belief very widely held by both the laity and the scientific worker is to the effect that the ingestion of distilled water is a bad procedure. The absence of inorganic matter in such water is believed to be the forerunner of various untoward influences upon the processes of digestion and absorption. So far as I am aware, there is no experimental basis for such a belief. One scientist (Findlay) says:

"If tissues or cells are placed in distilled water, passage of water into the cells occurs owing to the difference of osmotic pressure. The cells swell up and may finally burst and die. A similar poisonous action on cells is observed when distilled water is drunk. In this case the surface layers of the epithelium of the stomach undergo considerable swelling; salts also pass out and the cells may die and be cast off. This may lead to catarrh of the stomach."

If this scientist's claims are true, then one of our fasting tests is a notable exception. This is the fast which continued for over 100 days and to which reference has already been made (see p. 279). The fasting dog was given 700 c.c. of distilled water daily by means of a stomach tube, and yet at the end of the fast the post-mortem examination failed to show any evidence of a deranged gastric mucosa. Certainly a period of over 100 days is a sufficiently long interval in which to demonstrate the toxic influence of distilled water if such an influence is demonstrable. Particularly is this true of the fasting animal, which may possess a lowered resistance to toxic influences.

However, if we grant that distilled water, because of the absence of electrolytes, does possess a pernicious influence upon the gastric mucosa, it is quite logical to believe that such influence will be exerted to the maximum by distilled water taken *between meals*. Because of the electrolyte content of the average diet distilled water taken along with such a diet will cease to act as distilled water soon after it reaches the stomach. The toxic action of distilled water, if such action is demonstrable, must be more in evidence when the distilled water passes into the relatively empty stomach. So far as the swelling and ultimate bursting of the cells under the influence of osmotic forces is concerned, it must be apparent that osmotic phenomena which are exhibited by non-living, excised cells do not necessarily hold for cells actually functioning in the animal body. Distilled water in contact with a cell of the living body may, through osmotic influence, cause a swelling of the cell, but the actual bursting of the cell will, of course, be prevented by physiological factors which will be called into play, thus causing the circulation to remove the excess fluid.

Various clinical views have been expressed as to the influence of distilled water ingestion. Some clinicians claim to have found it harmful in certain instances, others claim it is harmless, while still others express the opinion that the question as to its harmfulness or harmlessness must be considered an open one. The catarrhal conditions which it is claimed follow the drinking of water from glaciers, or the excessive ingestion of ice, may possibly have had their origin in the low temperature rather than in the absence of electrolytes, although no untoward symptoms have resulted from the ingestion of ice water in the writer's experience (see below).

In our own experiments upon the influence of distilled water ingestion with meals (Bergeim, Rehfuss, and Hawk, Blatherwick and Hawk, Mattill and Hawk, Sherwin and Hawk), we were able to demonstrate a stimulation of the gastric and pancreatic functions, better digestion and absorption of ingested food, a decrease in the growth of intestinal bacteria, and a lessening of putrefactive processes in the intestine.

Ice Water.—When we come to ice water, we are dealing with a slightly different proposition since the question of temperature must be considered. In fact, the power of ice water to chill the stomach and to delay digestion is one of the main arguments advanced against the drinking of the cold fluid. In order to study this "terrible, chilling effect" of ice water, we had skilled mechanics construct a very delicate apparatus which enabled us to follow the temperature changes in the stomach while the food was actually being digested (Smith, Fishback, Bergeim, Rehfuss, and Hawk). And this is what we found. In twenty minutes after drinking a glass of ice-cold water (10° C.) the temperature of the stomach contents was approximately the same as that of the rest of the body. And in a like period of time, the temperature of hot coffee (50° C.) was also brought down to that of the stomach walls. It is truly wonderful how rapidly the stomach is able to regulate the temperature of the things we put into it, whether they be cold or hot! And the evacuation time is about the same for cold and hot drinks. Thus the "chilling effect" of ice water and the consequent delay in the digestion of our food is seen to be of no real significance under ordinary conditions. However, there is one time when we must use discretion in the drinking of ice water. That is immediately after vigorous physical exercise, and unfortunately that is just the time we feel like emptying the ice cooler. However, we must not do so for serious consequences may follow the drinking of large volumes of ice-cold fluid (water, soft drinks, etc.) at such times.

Conclusions

Before closing this discussion on water, the writer would like to emphasize the fact that, in all of the water studies made by his associates

and himself, normal subjects have been employed. We have made no clinical studies and have made no clinical suggestions. It *may be* true that a person with a deranged circulatory or gastric function, or any pronounced lesion of heart or kidney, should not drink large volumes of water at any time, either with meals or between meals. The ingestion of large volumes of water with meals *may be* contra-indicated in atonic or dilated stomach, since an excessive water ingestion might promote further atony and dilation. It *may also be* contra-indicated in gastroptosis, where the gastric support is relaxed and insufficient and in certain cases of pyloric colic and spasm. If contra-indicated in these conditions, however, *we have no experimental evidence to that effect,* and it is because a large volume or weight at any one time is contra-indicated and not because of the water *per se.* The writer would say, therefore, that normal persons may drink freely of water at mealtime, whereas those unfortunate individuals who possess lesions of heart or kidney or who are troubled with any circulatory or gastric disturbance, should have their fluid intake regulated strictly according to medical advice. The literature contains at least two observations (Marcus, Foster and Davis), indicating that the drinking of considerable water by nephritics causes no undesirable results, whereas the finding that the introduction of an excessive volume of fluid into the circulation causes no significant increase in blood volume or blood pressure (Bogert, Underhill and Mendel) would seem to indicate that patients suffering from cardiac disorders need not necessarily have their water intake materially restricted.

On the basis of a large number of experiments, made in the writer's laboratory and elsewhere, we feel warranted in concluding that the average normal individual will find that *the drinking of a reasonable volume of water with meals will promote the secretion and activity of the digestive juices, and the digestion and absorption of the ingested food, and will retard the growth of intestinal bacteria and lessen the extent of the putrefactive processes in the intestine. Furthermore, we would place no restriction upon the drinking of distilled water and none upon the drinking of moderate quantities of ice cold water, except when one is overheated following vigorous physical exercise.*

That Nature knew all these things long before we did is indicated by the fact that milk, Nature's best food, contains 87 per cent water and by the further fact that the birds and the beasts (Evvard) set man a good example to follow in the matter of water drinking at meals.

There is an old German proverb which reads "Alles Ubel vergeht durch Wasser und Diat." That is a perfectly good proverb, but I suggest that it be revised to read "Alles Ubel vergeht durch *reichlich* Wasser *in der* Diat."

The Metabolism of Alcohol

HAROLD L. HIGGINS

CINCINNATI

Introduction

Aside from the three important groups of foodstuffs, the proteins, the fats and the carbohydrates, ethyl alcohol, CH_3-CH_2OH, is the most available nutriment the animal organism has to meet its heat requirements. It is burned in the body to carbon dioxid and water, and each gram of alcohol when thus oxidized yields approximately 7.2 calories of heat. But while alcohol thus offers good possibilities from a nutritive point of view, its status as an altogether satisfactory food is enhanced by its pharmacological and toxicological action. This action of alcohol at first is most marked upon the central nervous system; the release of cerebral inhibition and the anesthetic features probably stand out foremost. The pathological changes as a result of overindulgence in alcohol are well known. It is quite universally recognized that too much alcohol is harmful to the human organism, and that, to be of any practical use for nutritive purposes, the quantity of alcohol taken must be small. Therefore, in discussing the nutrition of alcohol in this chapter the effects of moderate or small quantities will be more particularly considered.

Absorption of Alcohol

Alcohol requires no digestion for absorption, but it is absorbed directly from the gastro-intestinal tract mainly into the portal blood but also by the lymphatics (Dogiel, 1874). A considerable proportion of the alcohol taken by mouth is absorbed in the stomach and the remainder in the small intestine (Bodlander, 1883). The quantities or proportions absorbed in the stomach and in the different parts of the small intestine vary according to the rate with which the alcohol passes through the pylorus; alcohol taken with food will remain longer in the stomach and a larger proportion of it will be absorbed there than if the alcohol were taken on an empty stomach. One observer found that twenty per cent of alcohol was absorbed in the stomach, nine per cent in the duodenum, fifty-three per cent in the jejunum

and eighteen per cent in the ileum (Nemser, 1907). Alcohol is absorbed also when given by rectum (Carpenter(*b*), 1916) or when inhaled as vapor. Alcohol is not absorbed so rapidly when taken with food as without; fat especially seems to delay the absorption (Mellanby(*e*), 1919); the probable explanation for this is that absorption from the stomach is not so rapid as from the small intestine.

While alcohol does not require any digestion and is readily absorbed, it does influence the gastric digestion of other material (Kast, 1906). A dilute solution of alcohol increases the hydrochloric acid concentration without affecting the pepsin content of the gastric juice; less dilute solutions act as irritants to the stomach and cause increased mucus formation and often vomiting. But while alcohol may influence gastric digestion, yet the net effects on the availability of the fat, protein and carbohydrate in the diet is not interfered with; i.e., the amount of undigested residue in feces is not essentially different when alcohol is taken from when it is not (Atwater and Benedict (*e*), 1902). That is seen in the following table:

	Coefficients of Availability			
	Protein	Fat	Carbohydrates	Energy
Experiments	%	%	%	%
Without alcohol.....	92.6	94.9	97.9	91.8
With alcohol.....	93.7	94.6	97.8	92.1

The absorption of alcohol is rapid; this has been demonstrated (1) by the early psychological effects from taking the drug (Dodge and Benedict, 1915), (2) by its beginning to be burned in five to ten minutes after ingestion (Higgins (*a*), 1916), and (3) by increase in the concentration of alcohol in the blood (Mellanby(*e*), 1919). Very soon after taking alcohol (one-half to two hours), the blood will show the maximum concentration.

Excretion of Alcohol

From two to ten per cent of alcohol taken by mouth is excreted as such in the urine, the breath and the sweat (Atwater and Benedict, 1902; Voltz, Baudrexel and Deitrick, 1912). The remaining ninety to ninety-eight per cent is burned to CO_2 and H_2O. Alcohol is absorbed directly into the blood without chemical change, and is excreted in part unchanged by the kidneys, the lungs and the sweat glands. Alcohol is also excreted in the milk of nursing mothers (Nicloux(*a*), 1899). The amount excreted in the expired air and sweat is increased during muscular work, with the increased respiratory ventilation and sweating. The elimination of alcohol by the kidneys and lungs, also by the mammary glands, is by diffusion, the percentage of alcohol in the urine and milk

practically equaling that in the blood (Widmark (*a*), 1915; Nicloux (*b*), 1900).

Distribution of Alcohol After Absorption

The maximum concentration of alcohol in the blood is usually equal to or slightly higher than one would find if there were even distribution of alcohol throughout all the tissues (Mellanby(*e*), 1919). Analysis of various organs and tissues of the body after alcohol has been taken show that alcohol is quite equally distributed everywhere, but apparently there are some small differences, for the liver and heart muscle in rats have been reported as containing relatively low while the brain and blood contain relatively high percentages of alcohol (Pringsheim, 1908). This is shown by the following experiment:

Alcohol 5 c.c. per kilogram body weight given.

If equally distributed there would be 0.5 per cent throughout the body. There were found in the

Blood	0.52%
Brain	.41%
Kidney	.39%
Liver	.33%

The percentage of alcohol in the blood, or in the urine, should prove a good index as to the pharmacological and psychological effects to be expected; one observer states that intoxication does not appear unless the concentration of alcohol in the urine exceeds one-tenth of one per cent (Widmark (*b*), 1917).

Effects of Alcohol on Total Metabolism

Alcohol in moderate amounts does not increase the total metabolism of the human body (Atwater and Benedict (*e*), 1902; Zuntz and Berdez, 1887; Geppert(*a*), 1887; Higgins(*b*), 1917). Both the heat production and the heat elimination are essentially unchanged, for moderate quantities of alcohol cause no appreciable change in body temperature (Atwater and Benedict, 1902). However, large quantities of alcohol lead to marked peripheral vasodilatation with fall in body temperature; this is a cause of increased heat elimination, which in turn is followed by increased heat production as the body temperature returns to normal. Alcohol in being burned acts to replace some other source of energy and is neither a stimulant nor a depressor of the metabolism, and does not serve merely for "luxus consumption."

Effects of Alcohol on Protein and Purin Metabolism

Alcohol does not appreciably affect the protein metabolism; it neither acts as a protein sparer nor, unless taken to excess, as a protein destroyer (cell-poison) (Rosemann (*a*)). This is shown by determinations of the urinary and food nitrogen (nitrogen balance experiments). There is an increase in the nitrogen output and a negative nitrogen balance for about two days after alcohol is added to the diet; this is probably due to the change in the water balance of the body and non-protein nitrogen content of the body fluids and is associated with the diuretic action of alcohol; the nitrogen balance is uninfluenced by alcohol after the first two days. Some workers report that alcohol increases the uric acid excretion, while others have claimed that alcohol causes no change at all or an insignificant change (Rosemann(*a*); Mendel and Hilditch, 1910). Changes in the excretory action of the kidney rather than in the true uric acid metabolism seem to be the cause of the discrepancies found, and supplementary analyses to determine the uric acid content of the blood will be necessary to determine if the uric acid metabolism is affected by alcohol.

Combustion of Alcohol

Alcohol is burned by the body up to a certain percentage, when available in the tissues, in preference to either fat or carbohydrate. Experiments with men and animals show that the rate of combustion of alcohol is independent of the amount taken and comparatively constant (Mellanby(*e*), 1919; Voltz and Dietrich, 1912; Higgins(*b*), 1917). Over fifty per cent of the total heat production of the body seldom, if ever, comes from alcohol. When 30 c.c. of alcohol were taken by a man, the percentage of the total oxygen consumption used in burning alcohol during the first two or three hours was as high as when 45 c.c. were taken; about 20 to 40 per cent of the heat production (total metabolism) came from the alcohol, i.e. with a man in the resting state, about 3.5 c.c. of alcohol was burned per hour; thus if the same rate of combustion of alcohol continued (which is the case in animals) it would require 8 hours for all of 30 c.c. and 12 hours for all of 45 c.c. of alcohol to be burned (Higgins, 1917). The period during which alcohol will stay in the body when large amounts are taken is surprisingly long. Thus if a physician desires to give alcohol to a patient for its nutritive value, he should obtain as satisfactory results nutritionally and avoid many of the untoward features of alcohol, by giving it in small doses (10 c.c. or less), which may be repeated.

Alcohol displaces carbohydrate and fat acting to spare them. It probably displaces a larger proportion of carbohydrate than fat; i.e., if there

is a certain proportion of carbohydrate and fat being burned, and alcohol is ingested, it will be burned in preference to either up to about forty per cent of the total caloric expenditure of the body, and the ratio of carbohydrate to fat displaced in the combustion will be greater than the ratio of carbohydrate to fat previously being burned (Mellanby(*e*), 1919).

Alcohol and Muscular Work

While experiments have definitely proven that alcohol is burned in the body, and that it displaces carbohydrate and fat, but not protein, yet whether the potential energy of alcohol can be changed into the kinetic energy of muscular work in the body is still a matter of conjecture (Atwater and Benedict, 1902; Chauveau(*a*)(*b*), 1901). Experimental evidence is not at all conclusive, although it is generally believed probable, in the absence of evidence to the contrary, that alcohol can be converted into muscular energy. It is true that when alcohol is added to the diet of a person doing heavy muscular work, the work is not so efficiently nor so easily done (Van Hoogenhuyse and Nieuwenhuyse, 1913; Durig(*a*), 1906).

Definite and rather startling feats of endurance can be performed after alcohol is taken; thus one can hold the breath a longer time after taking alcohol than before, or one can hold on a bar longer or lift one's weight from the floor oftener at a given rate, etc. (McKenzie and Hill, 1910). A patient has been observed to be able to hold his breath fifty-three seconds before and one hundred and five seconds after alcohol (L. Higgins(*b*), 1917). This is probably to be explained on the basis of the dulling of the nervous centers by alcohol so that the brain does not react to fatigue so readily as normally, and it is not due to the energy yielded from the alcohol. But the fact stands out that alcohol gives one the power to perform certain feats of endurance of short duration.

Alcohol in Diabetes

Alcohol has been recommended in certain diseases, notably in diabetes. The diabetic person apparently can utilize alcohol much as the normal person, and can obtain a food value from it. Alcohol does not, however, act as an antiketogenic agent, i.e., in being burned, it does not act to prevent the formation of the acetone bodies as do carbohydrates (Higgins, Peabody and Fitz, 1916). However, if a diabetic has a change made in his diet so that a given amount of fat is substituted by an isodynamic quantity of alcohol, less acetone bodies will be formed in the body; i.e., alcohol does not form acetone bodies in its intermediary metabolism (Benedict and Torok, 1906).

Mineral Metabolism . . . . . *Henry A. Mattill and Helen I. Mattill*

Water—Sodium Chlorid—Alkalies—Calcium—Magnesium—Phosphorus—Iron—Sulphur—Iodin—Neutrality Regulation—Disturbances in Mineral Metabolism Accompanying Pathological Conditions.

Mineral Metabolism

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According to Albu-Neuberg the mineral constituents of the adult human body amount to 4.3-4.4 per cent. In this ash occur the elements Ca, P, K, S, Cl, Na, Mg, I, F, Fe, Br, Al, named in the order of decreasing amounts (Hackh). Any statement regarding exact amounts of the different elements is fraught with uncertainty for two reasons: first the paucity of reliable analytical data, secondly the individual variability due in part to differences in the organism, in part to differences in food habits and possibly to the existence of pathological conditions. The ash constituents of the new-born have been determined by Camerer and Söldner with results which show considerably more uniformity than do those on the adult. They find 2.10 to 2.73 per cent ash of which 38.5 per cent is P_2O_5, 36.1 per cent CaO, 9.1 per cent Na_2O, 7.8 per cent K_2O, 7.7 per cent Cl, 0.9 per cent MgO and 0.8 per cent Fe_2O_3. As compared with the adult these values are characterized by low total ash, CaO and P_2O_5, and by high Fe.

About 5/6 of the total ash occurs in the bones. Fresh bones contain about 35 per cent ash, about 84 per cent of which is $Ca_3(PO_4)_2$, 1 per cent $Mg_3(PO_4)_2$ and 7.5 per cent other Ca salts. About 99 per cent of the Ca in the organism is in the bones, about 70 per cent of the Mg and about 75 per cent of the P.

In a comparative study of the composition of the teeth of man and dog Gassmann(*a*) found 74-82 per cent ash. He was not able to recognize F. He found Ca and P most abundant, organic matter lowest, in the wisdom tooth, while organic matter was high and Ca and P low in the dog's tooth.

Cartilage contains only 1-6 per cent of mineral matter and its ash is higher in Na than that of any other tissue of the body, and is also characterized by a large amount of sulphates, which probably existed as organically combined S in the fresh tissue.

It may be safely assumed that the bone portion of the ash constituents is subject to less rapid metabolic changes than the remaining 1/6, of which the greater half is found in the muscles, the lesser half in the blood and

TABLE I

DENNSTEDT AND RUMPF

1000 Grams Fresh Substance Contain in Grams	Blood	Heart	Liver	Kidney	Spleen	Brain	Lungs
Water	806.17	798.86	797.85	837.98	860.83	820.11	901.4
NaCl	3.725	2.632	2.806	3.203	2.730	2.534	2.5
Na in addition to NaCl	0.40	0.422	0.533	0.457	0.326	0.545	0.032
K	1.338	1.610	1.718	1.643	1.691	2.450	0.836
Ca	0.123	0.150	0.124	0.174	0.173	0.137	
Fe	0.500	0.088	0.614	0.308	0.605	0.089	
Mg	0.100	0.122	0.145				
Total P	0.625	1.049	2.551	1.216	1.532	2.758	1.206
PO_4	0.994	1.864	3.241	2.434	2.518	....	1.643
Total S	1.575	1.544	1.775	1.397	1.643	1.32	1.095
SO_4	0.261	0.315	0.268	0.417	....	....	0.261

TABLE II

	100 gm. fat-free, dry substance contain			
	Cl mg.	Fe mg.	Ca mg.	Mg mg.
Muscle	302	125	33.2	106.4
Heart	769	39.6	46.8	102.9
Lungs	1421	372	92.3	40.9
Liver	529.8	335.5	39.7	96.6
Spleen	859	385.6	49.6	75.7
Kidney	1087.5	82.6	100.4	108.2
Intestine	525.4	114.6	116.3	63.7
Pancreas	933	26.1	92.2	97.4
Salivary gland	845	34.5	82.4	
Thyroid	848	29.0	169.4	48
Testicles	2545	56.9	93.6	107

other fluids, the nerves and organs. Dennstedt and Rumpf have made an exhaustive study of previous work on the mineral constituents of the different organs, and from this and their own work have compiled a table (I) giving what may be considered representative figures. These values are of interest chiefly in that they give an idea of the comparative abundance of the different elements, and they are to be considered as only approximately expressing the composition of any individual normal organ. There are no fixed relations in the ratio of different elements to each other, and variations amounting to as much as ½ to 2 times these average values may be found.

Recent work by Magnus-Levy(*j*) which is summarized in Table II is of special interest when compared with the values given above, for while the analyses are calculated to a different basis they allow comparisons of the relative amounts of the different elements, and show rather wide differences from the results of Dennstedt and Rumpf. That much of the normal variation may be due to variations in the fat and water content of the organs, components which may vary widely under physiological conditions, is very probable, especially in the earlier analyses. Magnus-Levy has eliminated these variables by calculating to a dry, fat free basis, and has probably eliminated variables due to pathological conditions, since his subject was a suicide. Pathological conditions are usually characterized by increased water and NaCl content, and by decreased Ca and P.

In the highly specialized cells the ratio of K : Na is higher than in supporting tissues (Gerard). In muscle, K phosphate is the predominant constituent and Mg is more abundant than Ca. As the result of analyses by Bunge, Aron gives the relationship of K : Na in muscle as 5-6 : 1. Benedict concludes that there is approximately three times as much Mg as Ca in the human muscle. Heubner found 0.15 per cent P in the fresh muscle of young dogs of which 70-90 per cent was water soluble (phosphates), 0.05 per cent P in the skin and 1.5 per cent P in bones. Meigs and Ryan have found the smooth muscle of the frog lower in K, Mg and

P, and higher in Na and Cl than the striated muscle. Since the K and P content of muscle is greater than that of the surrounding fluids, blood plasma and lymph, they conclude that the fibers of muscle are not surrounded by a semipermeable membrane, but that most of the water and of the K, P, S and Mg in the tissue is held in colloidal combination in a non-diffusable form. Many of the ductless glands are characterized by their rather marked content of one of the mineral elements in organic combination, as the spleen by iron, the thyroid by iodin and bromin (Labat), the hypophysis by P, the thymus by arsenic (Diesing).

Weil has recently studied the mineral constituents of human nervous tissue (Table III). If the concentration of these elements in the fresh nerve substance is considered, there is a rather interesting classification into two groups, the first of which, comprising Ca, Mg, P, S, Cl, shows wide variations in concentration, and the second of which, including Na, K, and Fe, maintains about the same concentration in the different nerve tissues. In view of the effect of the Ca concentration on irritability (see p. 336) it is interesting to note the lower concentration of Ca in gray matter. If the analyses are calculated to a water-free basis the conditions are reversed, the concentration of the first group is nearly constant, of the second group variable.

TABLE III

1000 gm. Fresh Nerve Substance Contains	Gray Matter	Cerebellum	White Matter	Spinal Cord
Ca	0.104	0.103	0.142	0.179
Mg	0.196	0.203	0.260	0.380
P	2.39	2.58	4.21	5.48
S	0.56	0.61	0.92	0.85
Cl	1.13	1.08	1.51	1.52
Sum (1-5)	4.380	4.579	7.042	8.409
Na	2.03	2.20	2.25	2.01
K	3.45	3.49	3.38	3.61
Fe	0.068	0.050	0.064	0.055
Sum (6-8)	5.538	5.740	5.694	5.675
Total (1-8)	9.918	10.316	12.736	14.084
Water	833	815	702	644

The understanding regarding the mineral constituents of the blood is even less satisfactory, and is subject to greater confusion than is that of the organs because in addition to the application of unsatisfactory methods, there has been confusion as a result of subjecting only a part of the blood, as the serum, the red blood corpuscles or the plasma, to analysis. Recent work is bringing order out of this chaos, with the result that the blood is coming to be looked upon as that constituent of the body showing most constant composition with respect to mineral constituents, under normal conditions (Table IV). From this it is not to be concluded that

TABLE IV

	1000 g. Normal Whole Blood Contain								
	H_2O g.	Cl g.	NaCl g.	K g.	CaO g.	Mg g.	P g.	S g.	Na g.
C. Schmidt[1]		2.620	4.318	1.739					1.902
Wanach[1]		2.588	4.265	1.813					1.989
Biernackl[1]		2.804	4.560	1.374					1.581
Denmstedt & Rumpf	806.17		3.725	1.338	0.123	0.100	0.625	1.575	
Jansen (*b*)					.115				
Austin and Van Slyke.			4.53						
Porte							0.57		
Bloor (*g*)							.30		
Courtney and Fales. infants' blood, 1-5 yrs.			in 1000 g. plasma						
	826.1		5.87						
Sakai, new-born	744		6.03						
Sakai, nursing infants.	814		5.81						
Sakai, adults	785		5.51						
Gettler and Baker	776		5.92						
						in 1000 g. serum			
Denis (*g*)						0.016-0.035			
				in 1000 c.c. serum					in 1000 c.c. serum
Kramer				0.16-0.22					2.80-3.10

[1] From Albu-Neuberg.

the composition of the blood is fixed, but rather that it varies within narrower limits than those for the composition of the organs.

Of the less abundant mineral elements Gautier has called attention to the wide distribution of F(*d*) and As(*a*) in the organism. F bears rather a striking relation to P; in the soft tissues and glands P : F is about 450, in the supporting tissue, bone and cartilage it is 125 and in the epidermis, hair and nails it is approximately 4. Injection of NaF into rabbits has been found to have an undesirable effect on Ca metabolism and F in foods is to be avoided (Schwyzer). Arsenic, Gautier found in the thymus and thyroid, in menstrual blood, in hair and skin. Bertrand confirmed these findings, which have been denied by others, possibly because organic As compounds would escape ordinary analytical methods. Van den Eeckhout found that ingestion of As promoted growth and well-being in animals. Bang(*f*) found that As in the urine varies greatly, depending on the amount in the foodstuffs, and may reach 0.5 mg. daily. Fish is especially high in As and on a fish diet he found 0.78 mg. As daily, while on a vegetarian diet the urine was As-free.

Silica is normally present in the urine and feces in amounts fluctuating with the intake (Schulz(*a*)). It is widely distributed in the body and comprises 40 per cent of the ash of hair and seems to be an essential constituent of the pancreas. Kahle calls attention to the loss of SiO_2 by the pancreas and its increase in the lymph glands of tubercular cattle, and to its increase in the pancreas in carcinoma. He found that the administration of the organic preparation of silica made by Weyland had a beneficial influence on the formation of connective tissue in the affected organs of tubercular guinea pigs. Schulz(*c*) considers that Kahle is not justified in his generalizations since there is a wide variation in the SiO_2 content of glands of tuberculous and carcinomatous patients. He found 0.0084 per cent SiO_2 in the normal dry thyroid and a larger amount in pathological thyroids(*b*). Gassmann(*b*) has identified selenium in teeth and bones. Mn (Reiman and Minot; Bertrand and Medigreceanu) is widely distributed in the human organism and is highest in the liver, averaging 0.17 mg. per 100 g. moist tissue. The blood contains 0.004-0.024 mg. Mn per 100 g., its function is probably catalytic. Small amounts of Cu and Zn are widely distributed in the body and always present in the urine and feces, their sources being undoubtedly the ingested foods (Van Itallie and Van Eck; Rost and Weitzer).

Older conceptions of the relative unimportance of salts for nutrition and the easy assumption that a normal mixed diet always supplied whatever need there might be for inorganic elements have recently given way to a recognition of the very definite needs of the body with respect to mineral constituents. Forster first established the fact that salt-poor diets led to faulty nutrition. What little work has been done on the ingestion of a salt-free diet leads to the conclusion that salts in the food are not pri-

marily necessary for the digestion or utilization of the foodstuffs, but that their lack even over a brief period leads to unpleasant nervous phenomena such as sweating, lack of appetite, listlessness and disturbed sleep and to fatal results if long continued (Lunin). Taylor(*b*) in a 9-day experiment on himself during which he ingested a ration consisting of 70-75 g. washed white of egg, 120 g. of fat and 200 g. sugar and containing less than 0.1 g. of salts, per day, noticed especially the nervous symptoms and a general muscular soreness. On the 9th day acetone was noticed in the breath, and acetone and diacetic acid in the urine, whereupon the diet was discontinued. The elimination of Ca and Mg through the urine ceased entirely after four days; Cl reached a minimum of 0.2 g. daily, phosphates were constant and conjugated sulphates were abnormally high; urinary ammonia rose only on the appearance of diacetic acid, suggesting that the fixed alkalies are required for the neutralization of the strong acids of S and P. Urinary acidity was constant. Diuresis and a loss in body weight (which was quickly regained on return to a normal diet) indicated a loss of water from the body. Goodall and Joslin repeated Taylor's experimental procedure on two subjects, and in both cases failed to confirm the appearance of either acetone or diacetic acid in the urine, although the nervous symptoms were similar, and they agree with Taylor in finding extremely low urinary chlorin, and considerable loss of weight due to a loss of body water. Unfortunately no complete study of the mineral balance was made and the opportunity which these conditions gave for throwing light on the fundamental mineral exchange in the body was lost. That the undesirable symptoms are in part though not entirely due to the acid-forming S and P present in the protein seems clear from the early work of Lunin, who found that Na_2CO_3 added to a salt-free diet prolonged the life of mice to about double its duration without the Na_2CO_3 but did not prevent death with the usual symptoms.

Fasting experiments have long been used to obtain fundamental information upon the metabolism of organic matter. The excretion of inorganic material during fasting gives similar information on mineral economy. In the study of prolonged fasting made at the Nutrition Laboratory of the Carnegie Institution (Benedict(*h*)) it appeared that the excretion of MgO (per kg. of body weight) was practically constant, especially after the first six days, and was about one third of the Ca excretion. There was a notable parallelism between the daily loss of Mg and of body protein although the Mg was always slightly greater than the calculated value from catabolized protein, using Magnus-Levy's figure of 0.106 per cent for the Mg content of dry muscle. Sodium elimination gradually fell during the first fifteen days, thereafter it was constant at a very low level (about 0.0011 g. Na per kg. body wt.) After the fifth day K_2O formed 80-90 per cent of the total alkali excretion (Na and K). If muscle has three times as much Mg as Ca and 5 or 6 times as much K as Na, mineral elimi-

nation in fasting cannot be regarded simply as waste products from protein catabolism. After 15 days Cl elimination was practically constant at 0.15 g. daily and was derived for the most part from disintegrated muscle substance. The ratio $\frac{N}{P_2O_5}$ was always lower than the accepted value for flesh, 6.6, the excess of P_2O_5 undoubtedly resulting from the metabolism of bones. Elimination of S was always less than would be expected from the ratio $\frac{N}{S} = 13.3$ in protein, and Benedict considers this an indication of the catabolism of some substance high in nitrogen and low in sulphur. The elimination of Ca and P, and to a less extent of K, in excess of that accounted for by muscle catabolism may be interpreted as an indication of a metabolic need for these elements which when not met by a proper intake is in normal cases met by the reserves in bone.

In their book published in 1906 Albu-Neuberg repeatedly deprecate the lack of sufficiently complete metabolism experiments to enable them to come to any reliable conclusions regarding the mineral requirements of the adult organism. Most of the work up to that time had been limited to the investigation of urinary excretion, and because of the lack of any approximately fixed relation between urinary and fecal output of Ca, Mg or P, was valueless. They point out that only by a painstaking investigation not only of the urinary output but also of the fecal output and of the food intake, can any reliable data regarding minimum requirements for normal conditions be obtained. Furthermore, in such controlled experiments, in which the intake is varied by the addition to the food of the mineral constituents sometimes in inorganic, sometimes in organic combination, another element of uncertainty is introduced in that the absorption and hence availability to the body of the minerals is not independent of the form in which they are ingested, and also the absorption of one mineral constituent depends to a degree on the quantities of other food materials ingested, *e. g.*, a condition of Ca equilibrium may be converted to a minus balance by the ingestion of an increased amount of P, of carbohydrate or of fat. We have only made a beginning in the acquisition of data which will finally lead to as definite an understanding of the mineral requirements as we now have of protein and energy requirements. With the recently attained success in feeding mixtures of purified foodstuffs to experimental animals has come a new method of determining the mineral needs. McCollum and Davis(*f*) have by this method shown that a ration in which the acid forming elements far outweigh the basic elements may support growth but is quite inadequate for reproduction. Osborne and Mendel(*e*) have varied the mineral content so as to reduce the quantity of one element after another, or of several at once, to a minimum, and they find that rats grow normally and equally well whether

deprived of Mg, Na or Cl or of all three. If deprived of K growth is not very satisfactory and when deprived of both Na and K it ceases. Lack of Ca or P is promptly followed by a slowing of growth.

Water

Of all the body constituents water is present in greatest proportion and except in the bones and fat it comprises more than one half the weight of the fresh substance. Three factors exert their influence on the water content of the body and of the individual organs. First, the age. The fetus has the highest percentage of water, at the third month 94 per cent, which falls rapidly so that by the fifth month it is approximately the same as at birth, 66-69 per cent (Camerer and Söldner). In the adult it is 58-63 per cent. Second, the nutritional condition of the organism. With poor nutrition the water content of the body increases, as a result of loss of fat, since water and fat are present in the tissues in quantities which vary inversely (Voit(*b*)). The ingestion of carbohydrates (Weigert(*a*)) and of NaCl favors water retention. Strauss(*d*) claims that for every 10 to 15 grams of salt retained 1½-2 kg. of water are retained, and he considers this a "sero" rather than a tissue retention. Third, a pathological condition is in many cases, especially in fibrile diseases, accompanied by water retention. Balcar *et al.* consider this to be the result of a poisoning of the tissues which causes them to combine with excessive quantities of water, thus interfering with regulation of body temperature by surface evaporation. By the injection of a solution containing 5 per cent NaCl and 1 per cent Na_2CO_3 until diuresis deprived the body of large quantities of water they were able to produce fever experimentally, and they compare this fever with the salt or inanition fever of new-born infants, both of which disappear on the administration of water.

Sakai's analyses of the blood of new-born infants as compared with that of nursing infants and adults show a lower percentage of water, and a higher percentage of salt in the new-born, $H_2O : NaCl = 122$, than in either of the others, $H_2O : NaCl = 140 — 142$. The maximum water content of the blood occurs at about three months of age and a too long continued liquid diet for babies is apt to prolong the period of high blood dilution with pathological consequences (Lederer; Widmer(*b*)). The normal water content of the blood is occasionally decreased in diabetes but pathological conditions usually result in its increase.

Edema is a water retention accompanied by salt retention which Fischer(*b*) considers the result of an accumulation of acid in the body (acidosis) since he has shown experimentally that increased H ion concentration promotes the absorption of water and of NaCl by protein. Henderson does not consider this explanation adequate because he finds no in-

creased colloidal swelling in H ion concentrations within the ranges that occur in the body or the urine, and because acidosis is not always accompanied by edema.

The requirement of the body for water is of course dependent to a degree on climatic and occupational variations, but under comparable conditions a child requires more water per kg. of body weight than an adult. Bartlett is of the opinion that a child 6 months old needs 122 g. water per kg. and an adult 35 g. Widmer(*b*) considers that a child 6 months old should receive 115 g. per kg.; a child 1 to 2 years old 65-110 g. water per kg. and that 85 g. is the optimum ingestion for a 2-year-old child. The daily loss of water through the lungs is 400-500 g. for adults. Lack of water, if accompanied by the ingestion of food, results in increased protein metabolism (Spiegler). A fasting animal is supplied with water for its body needs by the catabolism of its own tissues, and usually shows little inclination to drink. Excessive water drinking, in fasting or with food, causes temporarily increased N elimination followed by improved protein economy (Fowler and Hawk, Orr).

Sodium Chlorid

In how far sodium chlorid is a food and in how far it is a condiment, is a question which is open to discussion and which is not of particular importance. A certain amount of it must be considered a necessary food constituent for all but strictly carnivorous animals who suck the blood, as well as eat the flesh and bones of their prey, but there is no doubt that habit has resulted in the use of much more NaCl in the human dietary than is physiologically necessary. Albu-Neuberg state that 1-2 g. of NaCl daily is sufficient. While custom varies considerably the average daily intake is probably nearer 8-10 gr. Bunge's explanation that the need of NaCl by herbivora and animals living on a mixed diet is due to the preponderance of K over Na in grains, vegetables and flesh and that the absorption by the blood of the salts from these foods leads to a loss of blood Na and Cl which must be compensated by ingestion of NaCl, is still generally accepted. According to this theory the K and Na salts from the food enter the blood as organic salts or as phosphates and since the ratio of K to Na is higher than in the blood, the excess of K salt reacts with NaCl in blood, producing KCL and a Na salt, both of which are excreted by the kidneys thereby impoverishing the body of NaCl. Köppe has added to this the theory that salt hunger may be due to a lack of ionized salts in vegetable foods.

The relation of salt to water retention has already been mentioned (p. 311). This matter has been attacked experimentally from different directions with interesting results. Cohnheim and his co-workers have shown

that the water lost on profuse sweating is much more rapidly regained on a salt-rich than on a salt-poor diet, when water and food intake are otherwise unchanged. They hold that the large amount of dilute urine following muscular exertion is due to the thirst which prompts water drinking and since no salt is taken with the water it cannot be incorporated into the body. The fact that thirst is only transitorily slaked by water drinking under such conditions is also a result of the lack of NaCl.

Working from the other direction Belli reduced his NaCl intake to a minimum during 10 days of a metabolism experiment which consisted of 4 days preliminary period (10.2 g. NaCl daily) 10 days salt-poor (1.03 g.) and 3 days final (9.32 g.). His decreased water intake during period II (2000 g.) was enough to account for his loss of weight (1.3 kg.) since water excretion was practically unchanged, and in the final period he rapidly regained weight with water balances as follows:

	Water Intake	Water Loss in Urine and Feces	Body Weight, Kg.
Last day, period II.....	2102	1517	64.8
1st day, period III.....	2279	950	65.6
2nd day, period III.....	2292	1327	66.2
3rd day, period III.....	2087	1833	66.2

During period II the urinary Cl fell to 0.04 per cent and in the last five days there was Cl equilibrium. Klein and Verson in 1867 found a similar loss of weight in a period without salt and in the following period a large gain which they ascribed to water retention.

In experimental work on a diet free from all mineral constituents similar losses of weight have been followed by a rapid gain, in one case 4.1 kg. in 72 hours, on a return to a normal diet or on the addition of only NaCl (Taylor(*b*); Goodall and Joslin).

There is apparently no continuous storage of NaCl in the body, an increased intake may result in slight retention for a few days, but equilibrium is soon established on the higher level. In work on dogs v. Hoesslin established that on an intake sufficient to exceed the minimum needs all the ingested NaCl was eliminated by the kidney, not equally on all days but with daily and periodic variations. On a quantity of salt much exceeding the minimum needs there was likewise equilibrium over a long period, but from day to day the capacity of the organism for water and salt varied within limits which were about 10 per cent each way from the average. The water content of the feces is less the greater the salt intake, Cl and water secretion by the kidney run approximately parallel.

Urinary elimination of Cl undergoes a rapid rise upon ingestion of food (Dobrovici; Hermannsdorfer), due to absorption of NaCl by the stomach, followed by a fall representing secretion of HCl in the gastric juice, which is accompanied by increased alkalinity of the blood (Van

Slyke, Cullen and Stillman), and then a slow rise representing absorption from the intestine.

On a salt-free diet and in fasting the salt elimination soon falls to a very low level, below 0.3 g. chlorin daily, and remains there. It is impossible to lose more than 10-14 per cent of the body chlorids and Rosemann has shown that the body husbands its supply of chlorids so thoroughly that only by removal of the HCl of the gastric juice by fistula or stomach tube can symptoms of Cl hunger and malnutrition be produced. The ingestion of NaCl after fasting is followed by retention for a few days and then the equilibrium is reëstablished. Recent work indicates that the skin is an important storage place for chlorids (Padtberg(*a*); Wahlgren).

Early work on the influence of NaCl on metabolism led to the conclusion that it stimulated protein metabolism but later work on sheep, dogs and men has proven that moderate quantities of NaCl act as a protein sparer (Belli) reducing the N elimination 2-6 per cent without affecting the total energy exchange. Pescheck (*a*)(*b*) has shown a similar protein sparing action of Na acetate, citrate, lactate and Mg acetate, in some cases accompanied by diuresis. The ingestion of NaCl increases the renal and decreases the intestinal elimination of Ca, probably without changing the total excretion (Towles; v. Wendt(*a*)).

The blood is characterized by a greater constancy in NaCl concentration than is any other body constituent (Biernacki, Gerard). In children the plasma NaCl varies between 0.536-0.626 per cent, avg. 0.587 per cent, and in disease it is usually below normal. Veil found that in adults the plasma NaCl varied between .575 and .637 per cent with an average of 0.61 per cent. The corpuscles contain about 40 per cent as much as the serum (Snapper(*b*)). Authorities differ as to the influence of the diet, Veil found plasma chlorids decreased on a salt-poor diet, increased on a salt-rich diet, Arnoldi(*b*) found the opposite unless a large ingestion of water accompanied the high NaCl intake, when chlorids might be increased. Austin and Jonas found chlorids independent of diet and Barlocco found that the administration of NaCl *per os* resulted in a transitorily increased concentration of blood salt followed by a decrease which continued until compensated by kidney activity, when it again increased; while intravenous injection did not produce the preliminary rise, but caused reduced NaCl concentration followed by a rise unless nephrectomy had been performed. In view of recent findings on the tendency of the organism to maintain constant blood volume and concentration (Bogert, Underhill and Mendel; Smith and Mendel) the question deserves further investigation. Gastric secretion does not appreciably affect blood chlorid concentration (Rosemann(*f*)). Ingested salt seems to be without effect on the gastric secretion judging from the work of Rosemann and from the normal food utilization found in salt-free diets. On the other hand

there is evidence that loss of salt through excessive perspiration leads to hypoacidity (Cohnheim and Kreglinger).

Work by Frouin and Gerard on a dog with Pawlow stomach may bear upon this. Having usually received 10 g. NaCl daily, the dog was reduced to a salt-free diet of 200 g. rice and 700 g. horse meat cooked in water with the following results:

	NaCl Intake	Gastric Secr. 24 Hrs.	Acidity as g. HCl per liter	Total Chlorids as g. HCl per l.	K per liter	Na per liter
Jan. 12	0	350 c.c.	2.81	5.55		
13	0	275	3.32	5.57		
14	0	115	3.28	5.67		
15	0	113	1.97	5.57		
16	0	96	1.38	5.84	0.15	2.21
17	5 g.	185	3.39	5.98		
18	5 g.	190	3.06	5.39	0.22	0.96
19	0	90	1.20	5.90		

which are striking for the constancy of the total chlorid content and the decreased acidity of the secretion with lack of NaCl in food. The ingestion of a chlorid, whether NaCl, KCl or $CaCl_2$ brought the quantity, acidity and concentration of Na and K in the gastric juice back to normal. Batke found a similar decreased gastric acidity in salt hunger.

Since ingestion of acids causes loss of alkalies from the body the Na and K elimination in hypo- and hyperchlorhydria has been the subject of some investigation, and has been found to be unaffected by such gastric disturbances (Secchi(*b*)). Blood chlorid in hypoacidity may be higher than in hyperacidity (Arnoldi(*a*), Strauss(*c*), Veil). However, in diseased conditions which affect kidney permeability, notably in nephritis, high blood chlorids usually occur and at the same time hyperchlorhydria—the stomach apparently taking on the excretory function which the kidney has lost (Goyena and Petit; Crosa).

Alkalies

The alkali metals Na and K are present in all organs and tissues. Those tissues having important functions, and which are rich in cells have a higher ratio of K to Na than the tissues of conduction and support or the body fluids but there is no absolute specificity between Na and K in any organ, and the blood alone, of all the tissues and fluids, conserves its ratio of Na : K in spite of régime or food. The ratio of K : Na is highest in the vertebrates and is normally about 2½ : 1.

This difference between the quantities of Na and K in the body is reflected in most foods especially in milk and vegetables, and in infancy the retention is in approximately the same ratio as the occurrence in human milk (Cronheim and Müller(*c*), Meyer(*b*)). In the usual mixed diet the ration of Na : K is reversed, because of the addition of NaCl

to the food and what little metabolism work has been done on alkali balance, does not give conclusive results regarding their retention chiefly because the loss of the alkalies, especially Na, through sweat makes the determination of total excretion difficult.

An abnormally high ratio of K : Na (22:1) in the food of puppies has been shown to result in a strong positive K balance and a slightly negative Na balance, and when long continued, to stop growth. The ratio of K:Na in the liver and kidney was 1.5 to 1 while in control animals (receiving K:Na 2:1) it was 1.24 : 1 and in rats a very high K diet brought the ratio of K : Na in their ash up to 2.41 : 1, when it is normally 1.5 : 1 (Gerard(*b*)). Osborne and Mendel(*l*) have found K more essential than Na in the diet of rats. The bones of calves receiving a high K diet showed retarded development even with a plentiful supply of Ca and P_2O_5 in the diet, though the composition of the bones was normal (Aron (*a*)). An effort to confirm these results on children by studying the CaO balance on diets high and low in K (K: Na 2: 1 and 1: 17) has been unsuccessful (Adler).

The ingestion of a diet rich in fat affects the alkalies in the same way that it affects Ca, and may lead to a negative balance (Hellesen). Ingestion of acids has a similar effect (Secchi(*a*)). Elimination of the alkalies is principally through the urine. The feces usually contain more K than Na, but only in cases of diarrhea does the quantity of either become a considerable proportion of the total excretion. There are 3-4 grams K_2O, 5-8 g. Na_2O daily in the urine of the normal adult, though these quantities are subject to wide variations depending on the diet. In starvation the elimination of Na rapidly decreases, of K less rapidly, and after a few days the K elimination is six to nine times as great as the Na, a proportion which exceeds that found in muscle substance. On breaking a fast and in convalescence there is a very marked K retention.

The coincidence of glycosuria and acidosis has resulted in the development of an alkali therapy in diabetes for which a considerable success is claimed (Underhill(*g*)). In opposition to this claim must be mentioned the findings of others, that $NaHCO_3$ administration is sometimes followed by retention of chlorids and water resulting in edema, and that the apparently improved carbohydrate utilization may be only a result of its storage in the increased body water (Levinson; Hertz and Goldberg; Beard).

Calcium

The distribution of CaO between urine and feces is too variable to permit of any even approximate statement. The urinary CaO may comprise 5-64 per cent of the total CaO excreted in the normal cases (Neurath, Towles). A milk diet is apt to result in a lower proportion of urinary CaO to total CaO than a mixed diet (Secchi(*b*)) in spite of the fact that

urinary CaO is higher on a milk diet than on a mixed diet; and milk is more effective than Ca lactate in increasing urinary CaO (Givens(*b*)). Breast-fed infants usually show higher urinary CaO, in terms of per cent of total CaO, than the artificially fed. NaCl and HCl increase the per cent of urinary CaO but do not affect the Ca balance (Givens(*b*), v. Wendt(*a*)) while bases are without effect (Givens) except in pathological conditions (Eppinger and Ullmann). An increased urinary CaO is usually accompanied by diuresis (Schetelig).

Calcium in the food is usually in organic combination, as in milk, eggs, vegetables and cereals, though there is a not unimportant intake of lime from drinking water, in inorganic combination. The question as to the relative availability of these two forms has not yet been settled (Bunge(*d*); McCluggage and Mendel; Rose (*b*); Aron and Frese). Givens found that 0.34 g. CaO in the form of dried skim milk when added to a Ca poor basal ration would produce a positive Ca balance, while 1 g. of CaO in the form of lactate was necessary to accomplish the same end. In two cases of exophthalmic goiter Towles found that the addition of Ca lactate to a Ca poor diet which was giving a negative balance, resulted in a positive balance which soon reverted to negative, while the addition of the same amount of CaO in the form of milk gave a higher and a lasting CaO retention. That inorganic Ca salts, especially the soluble ones, are absorbed is indicated by Kost who found notable increased Ca in the bones of rabbits fed $CaCl_2$ for a long period, as compared with control animals. Orgler, supplying Ca in the form of Ca phosphate, found equally good retention whether the salt was given in raw milk or in sterilized milk.

The adult normal requirement for Ca has been variously estimated 3.3 g. (Bunge) to 0.38 g. CaO per day. Bertram maintained equilibrium on 0.38 g. CaO. Renvall required 1.19-1.26 g. CaO. Von Wendt(*a*) considers 0.8 g. CaO daily sufficient and Nelson and Williams by studying the total elimination of four subjects on normal unrestricted diet found 0.95-1.43 g. CaO excreted daily. Sherman(*c*) considers 0.9-1 g. CaO per day sufficient, since it is considerably above the average amount found by him in a compilation of 97 experiments in which a minimum CaO for equilibrium was determined (0.63 g. CaO per 70 kg. body weight)(*e*). He states "the case of Ca is the one which would seem to call for the most liberal margin in intake over the estimated average maintenance requirement if individual variability is to be covered by an ample factor of safety." He holds that 1 g. of Ca should accompany every 100 g. of protein intake. A sufficient Ca supply is so important that some investigators have recommended the addition of Ca salts to bread and others the direct ingestion of 1 to 1.5 g. $CaCl_2$ or Ca lactate daily (Heinze; Bertram; Loew). Such an addition does not affect the arteries (Kost) and has been shown in animal experimentation, to have beneficial results (Emmerich and Loew(*b*); Evvard; Dox and Guernsey). Pellagra producing diets have been shown to

be deficient in Ca (McCollum, Simmonds and Parsons). The ingestion of excessive quantities of fat, protein or carbohydrate increases lime excretion (Kochmann(*a*)(*b*)). N and Ca balances show no parallelism whatever.

Albu-Neuberg state that NaCl increases and that alkalies reduce CaO resorption: neither v. Wendt nor Givens support this statement. Aron found that high K and low Na intake decreased Ca absorption, but Adler was not able to confirm this. Dubois and Stolte by adding alkali to the diet of rachitic children were able to convert a negative to a positive lime-balance, but if the balance was originally positive the addition of alkali had little effect. Neither Givens nor Granstrom were able to show any effect of alkali or acid administration on the lime balance of a dog. Secchi on the other hand found in dog and man an increased Ca output, especially in the feces, when HCl was administered. Undoubtedly the nutritive condition of the individual at the time such an experiment is initiated influences the result; Givens' dogs were on a minimum or even inadequate Ca intake, while Secchi's subjects showed a positive Ca balance. An addition of H_3PO_4 causes an increased CaO output in both urine and feces.

In the adult there is a tendency to Ca equilibrium. Renvall increased the lime intake over the amount necessary for equilibrium by ingesting $CaCO_3$ and found a retention of CaO for several days, followed by equilibrium on a higher level of intake and output. This is strikingly like protein and NaCl metabolism, and is confirmed by Sherman and by Herbst.

In infancy and childhood the question of lime metabolism, as of phosphorus, becomes one of especial importance because of the need of the body for these elements in growth and especially in bone formation. Weiser has shown in work on dogs that gain in weight, on a diet poor only in Ca, is below normal, and surprisingly enough, the bones make up a larger percentage of the total body weight than in the control animals. The water content of the bones was 20-30 per cent higher than that of the controls, the ash content lower, and the fat content about the same. The composition of the ash varied from the normal and the variation was greatest in the ribs and least in the skull, and was characterized by decreased Ca, P_2O_5 and SO_3, and by the appearance of 3-5.5 per cent Na_2O and 0.35-1.25 per cent K_2O. Aron and Sebauer confirm this. E. Voit found the breast bone and skull of pigeons to be most affected by a Ca free diet. Aron(*d*) and Brüning in work on growing rats which they maintained at constant weight by underfeeding on an otherwise adequate diet, or by food containing only carbohydrate, found a markedly increased percentage of ash in the total body, as compared with control animals of the same weight but younger.

The amounts of the mineral elements required to make a gain of 100 g. in the body weight of infants have been calculated from various angles. Camerer and Söldner based their estimate on the composition of new-born

TABLE V

Grams Going to Make 100 g., Gain in Weight	K_2O g.	Na_2O g.	CaO g.	MgO g.	P_2O_5 g.
Camerer and Söldner ..	0.20	0.24	1.00	0.04	1.04
Cronheim and Müller					
3-4 months old	1.53	0.66	1.97	0.18	1.77
5-6 months old	1.26	0.49	0.48	0.12	0.78
Meyer	0.73	0.17	0.3	....	1.17
Tobler and Noll, 2½ months old	0.69	0.82	0.21	....	0.47

infants, Cronheim and Müller on the retention found in metabolism experiments extending over 35 days, and Meyer on the metabolism of fasting. Tobler and Noll report a metabolism experiment on a 2½ months old baby giving the average retention per day on an average daily gain of 24.3 g., and for the sake of comparison their values for retention have been multiplied by 4, to make approximately a 100 g. gain in weight, and are included in Table V. Bartlett's estimate that 1.7 g. ash must accompany every gram of N laid down is probably within these limits. He considers 0.05-0.8 g. Ca per day a normal deposit; Herter considers 0.1 g. CaO the daily deposit necessary for normal growth. Apparently gain in weight is due to such variable proportions of bone, protein, water and fat that only an approximate estimate of the mineral need can be made on this basis. Children 6-7 years old should get 0.3-0.5 g. CaO per day, 14 years old, 0.6-0.9 g., in order to support normal growth of bones (Herbst).

It is generally conceded that human milk contains the mineral constituents in the ideal proportions for growth, although Dibbelt and Aron(*b*) point out that the breast-fed baby's need of lime may exceed its supply in the first six months of life, and thereafter the supply exceeds the need. In this connection it is worth while to refer to recent very painstaking analyses of woman's milk by Schloss(*a*) and Holt(*b*) and of cows' milk by Trunz who show a colostrum period consisting of the first 12 days and characterized by high ash content, a transition period to the end of the 4th week after which the composition remains about constant until the 10th month. This can best be summarized, and the difference between human and cow's milk displayed in the following table (VI). Schloss compared the complete 24-hour samples of milk from 8 wet nurses and found a marked parallelism between the N and total ash. The lower content of Ca in human milk is compensated by a much better absorption.

The feeding of vegetables to young babies (6-7 months old) has recently been shown to exert a favorable influence on their growth. The increased quantity of salts, their especially favorable chemical nature, or the vitamin content are variously suggested to explain this effect. Since boiling vegetables in water causes a considerably greater loss of salts than steaming, the latter method of cooking is recommended (Courtney; Fales and Bartlett).

TABLE VI

G. in 100 cc. Milk.	Ash	CaO	MgO	P_2O_5	Na_2O	K_2O	Cl
Human Milk							
Holt—colostrum ..	0.3077	0.0446	0.0101	0.0410	0.0453	0.0938	0.0568
Holt—early mature, 1-4 mos.	.2056	.0486	.0082	.0342	.0154	.0539	.0351
Holt—middle mature, 4-9 mos. ...	.2069	.0458	.0074	.0345	.0132	.0609	.0358
Schloss—mature ..	.1839	.0376	.0086	.0405	.0189	.0529	.0522
Cows' Milk							
Trunz—colostrum...	.766	.194	.027	.238	.052	.174	.092
Trunz — mature, period II	.714	.174	.019	.205	.042	.176	.101

Aschenheim(*b*) found that the addition of fat to the diet of infants increased the fecal CaO at the expense of the urinary and that if the child was sick or convalescent the drain on CaO might be so great as to establish a negative balance. Meyer and Birk and Rothberg found a like effect of fat on the balance of Na, K, Mg, and Ca. Herter showed that the loss of CaO in infantilism was connected with poor utilization of fat, and the excretion was in the form of a Ca soap. He also concluded that a small increase of fat in the food might convert a positive CaO balance to a negative one. Recent work (McCrudden and Fales) has not substantiated Herter. Niemann(*b*) in a metabolism experiment on a normal 10-months' old infant varied the fat content of milk from 1.13 per cent to 3.97 per cent and found a constant excretion of CaO throughout, on an intake of 1.8 g. CaO per day. He concludes that in normal infants the change from a fat-poor to a fat-rich diet, so long as the fat content remains within physiological limits, does not interfere with CaO absorption and does not increase the fecal CaO although the typical fat stools are present. Others confirm this (Wolff; Holt, Courtney and Fales(*d*)). Hoobler(*a*) goes even further and shows that a high fat content if within normal physiological limits favors retention of Ca and P but this is not the case if the fat rises above the normal quantity in human milk (Lindberg). For infants on modified cow's milk Holt and his co-workers found the best absorption of Ca when the food contained 0.045-0.060 g. CaO for every gram of fat and when the fat intake was not less than 4 g. per kg. body weight. For young children on a mixed diet the absorption was best when the fat intake was not less than 3 g. per kg. body weight and there was 0.003-0.005 g. CaO to every gram of fat.

In artificial feeding with cows' milk the intolerance for fat often noticed may be caused by the excessive amount of calcium present which for lack of sufficient Cl or phosphate for its excretion as a salt of either of these acids may be excreted as a Ca soap or may accumulate in the tissues causing fever and finally being excreted as Ca lactate. The dilution of the milk with whey, thus supplying a large proportion of acid elements, or

"decalcifying" of the casein improves the fat and mineral utilization in such cases (Bosworth, Bowditch and Giblin; Bosworth and Bowditch; Forbes(*c*); Giffhorn).

The mineral requirements of childhood and adolescence have been subjected to metabolism studies by Herbst(*a*) and Jundelt with the following results:

	Herbst	Jundelt (2 boys)	
	(6 boys—6-13 yrs.)	5½ yrs.	7¾ yrs.
P_2O_5 retention per kg. body weight per day..	0.027—0.037 g.	.0315	.0297
CaO retention per kg. body weight per day..	0.01 —0.02 g.	.0029	.0294
MgO retention per kg. body weight per day..	0.002—0.007 g.	.0140	.0159

In another 12-day study of two rapidly growing adolescent boys Herbst (*b*) found a daily exchange per kilogram of body weight as follows:

A	CaO Retained g.	CaO Excreted g.	P_2O_5 Retained g.	N Balance g.	
Subject I.	0.0075	0.0146	0.0148	+ 0.013	6 days of muscular exertion
	.0042	.0204	.0138	+ .045	6 days of rest
Subject II	.0118	.0075	.0039	— .020	6 days of muscular exertion
	.0093	.0128	.0111	+ .029	6 days of rest

These values are of interest in showing the relation of CaO deposit to bodily activity and the lack of any parallelism between CaO and N. Hoppe-Seyler and v. Noorden have noticed increased CaO elimination in bodily inactivity.

Recent work has greatly extended our information regarding the calcium of the blood. That calcium, though present in small amount, is one of the important constituents of the blood because of its effect on coagulation and heart irritability, has long been acknowledged. We are indebted to Jansen(*b*) for a review of previous work, the development of an analytical method and analytical results. Previous investigators have found 4.0 to 11.9 mg. CaO per 100 c.c. blood (using strictly chemical methods), with variations for a given species as great as the difference between various species. Semi-exact methods, devised by Blair Bell and Wright, have resulted in such wide variations in findings when employed by different investigators (Katzenellenbogen; Morley; Mullik) that these results will not be considered in the following summary. Jansen, Voit, Dhere and Grimme, and Dennstedt and Rumpf agree in finding a variation in blood calcium dependent on age and independent of sex. At birth the infant's and mother's blood are about the same in Ca

content. The Ca in infant's blood increases during several months after birth; it reaches a maximum which varies but may be as much as double that at birth, and thereafter there is a gradual decrease. Jansen in the analysis of the blood of 33 men and women found an average of 12.46 mg. per 100 c.c. of whole blood at 20-30 years of age, 12.25 mg. at 30-40 years, 11.3 mg. at 40-50 years, and 10.95 mg. above 50 years. Dennstedt and Rumpf found 11.6 mg. the average of many determinations on adults. Using a nephelometric method Lyman(*a*) found about half this amount, and slightly higher in women than in men. There is a difference of opinion regarding the distribution of the blood Ca between the plasma and corpuscles, some (Lamers) considering that all the Ca is in the plasma, others (Heubner and Rona; Cowie and Calhoun) that it is in both plasma and corpuscles. Jansen found that if he washed the corpuscles free from plasma with isotonic sugar solution they usually contained some Ca (1-3.5 mg. CaO per 100 c.c. whole blood), but if they were washed with hypotonic NaCl solution they were free from Ca, and he concluded that the Ca is dissolved in a diffusible form in the corpuscles. Heubner and Rona found a similar distribution between plasma and corpuscles in cat's blood. The fibrin, Jansen found, contained 0.34 mg. CaO per 100 c.c. whole blood. The Ca content of the cerebrospinal fluid is about half that of the blood and is less subject to fluctuations in pathological conditions (Halverson and Bergeim).

The calcium content of the blood during pregnancy and lactation has been the subject of considerable investigation because of the unusual drain on body Ca at such times. During pregnancy and the puerperium Jansen found an average of 12.5 mg. CaO per 100 c.c., whole blood, a normal value for the age. Lamers found 0.8-1 mg. higher CaO in pregnant and lactating women, but he found high blood CaO in women 4-8 weeks after delivery, regardless of whether they were lactating or not. Possibly this illustrates the lag in adjustment after pregnancy which McCrudden considers an explanation of osteomalacia (see p. 339). Lamers and Mullik suggest that a rise in blood CaO causes the onset of labor. The ingestion of a Ca-poor or Ca-rich diet or of Ca salts seems not to affect the blood Ca (Clark; Denis and Minot(*h*)).

The important rôle which the Ca ion plays in controlling the permeability of colloidal membranes leads Brinkman(*b*) to the conclusion that the Ca ion concentration of the blood is as constant at H ion concentration, and that the distribution of the Ca in the blood between a protein compound (25 per cent) and Ca $(HCO_3)_2$ and its ions (75 per cent) supplies the necessary mechanism for its adjustment. Rona and Takahashi place this Ca ion concentration at 30 mg. per liter of serum. The increased blood calcium which has been found on subcutaneous injection or inhalation of $CaCl_2$ (Clark; Heubner and Rona) and which Voorhoeve claims to have found on ingestion of large amounts of Ca in food, cannot

(according to Rona and Takahashi) affect the Ca ion concentration of the blood to any degree.

Magnesium

Magnesium has not so far taken on the importance that the other minerals have in a consideration of mineral metabolism, possibly because the body need is relatively small and always sufficiently covered by the food supply so that the nutritive disturbances which might follow lack of Mg are not observed. Osborne and Mendel found that a diet poor in Mg supported growth of rats as well as one richer in Mg but in the Mg-poor diet they may not have gotten below the minimum requirement. The very small amount of Mg in human milk, which is not compensated by a storage in the infant's body as is Fe, leads to the conclusion that Mg needs are at least extremely low. Bertram found that 0.73 per day more than covered the body needs, and resulted in storage of Mg for a few days, after which equilibrium was established. Renvall found a balance established on an intake of about 0.45 g. Mg; on 0.25 g. there was a loss of Mg by the body. Von Wendt(*a*) found in one case a slight storage on 0.20 g. MgO daily and in another a loss of Mg on 0.33 g. Sherman in studies on 150 American dietaries found an average intake of 0.34 g. Mg per day, which probably expresses a little more than the minimum requirement. Neither Mg (Wheeler) nor Sr (Lehnerdt) can replace Ca physiologically.

In bones the amount of Ca is 8 to 9 times that of Mg, in muscle the Mg is 2 to 3 times the Ca, in nerves the amount of Mg is about twice that of the Ca. In fasting the elimination of Ca is 3-4 times that of Mg, indicating a catabolism of both bone and body protein.

Absorption of Mg is similar to that of Ca, though it seems to suffer less interference by the presence of other substances. Its distribution in the urine and feces is subject to the same variations as that of Ca under similar conditions though a larger proportion of the total Mg is urinary; urinary Mg is usually lower than urinary Ca (Givens(*b*)). The ingestion of large amounts of Mg salts has been found to increase the Ca elimination, but Mg elimination seems to be independent of Ca ingestion (Malcolm; Hart and Steenbock(*a*). Fats and carbohydrates decrease Mg retention in infants (Birk).

Phosphorus

None of the other inorganic elements has so wide a distribution in various forms in the animal body as has phosphorus. Its importance in life processes is reflected in the great volume of literature that has been contributed upon its occurrence, its nutritive history and its functions.

A compilation and review of the information available in 1914 forms a compendious monograph embracing about 3,000 titles, and it would seem unnecessary, indeed, if not impossible to refer individually even to the more important contributions before that time (Forbes and Keith).

In inorganic form phosphorus is found in animal and plant tissues chiefly in the form of K and Ca salts of phosphoric acid and in the organic forms in the generally familiar classification as nucleoproteins, phosphoproteins and lecithoproteins or phosphatids. To these should be added the phosphoric acid esters of carbohydrates and related substances which may be found increasingly important as investigation continues; for example, a phosphorus-containing carbohydrate is regularly found as a constituent of starch (Northrup and Nelson).

The distribution of the different forms of P in the organs and tissues has claimed the attention of several investigators recently and the resulting outstanding facts are that inorganic phosphates make up the greater amount of muscle, bone and blood phosphorus (Heubner; Greenwald(*f*), that the important substance for muscular activity is a compound of lactic and phosphoric acids which is derived from organic P compounds (Embden), that in smooth muscle the protein P is more abundant than in striated (Costantino), that lack of P in the food affects first the inorganic P of the bones and liver and that of the other organs only very gradually. The brain and heart lose total P under no conditions of dieting (Masslow(*a*)), exceptional ingestion of P as phosphates seems to decrease the P content of the central nervous system, although it does not seem to influence the deposit of phosphatids in muscle and bone, the percentage of which is remarkably constant throughout life; possibly it does affect the nucleoproteins (Heubner).

An estimate of the phosphorus requirement is rendered doubly difficult because of the uncertainty which surrounds the question of the availability of the different forms of phosphorus in foods. Unquestionably there is a difference between the phosphates and the organic P compounds both in the rate and the percentage of absorption. Experimental studies in which phosphates have been added to a diet poor in P can therefore hardly be compared with those in which an ordinary mixed diet has been used. Sherman found from a study of 95 balance experiments that the minimum requirement averaged 0.88 g. P per day per 70 kg. body weight, and he considers 3.50 g. P_2O_5 per day a sufficient intake. Berg maintained equilibrium on 2.25 g. P_2O_5 daily at the same time that Ca equilibrium was maintained on 0.33 g. CaO, and he showed that the addition of 10 g. $CaHPO_4$ to this diet not only resulted in no retention of either P or Ca, but caused a loss of Ca from the body. Von Wendt on the other hand was able to convert a negative CaO balance to a positive balance by the addition of 3g. $CaHPO_4$. Any definition of the P requirement without at the same time taking into consideration the Ca supply, or vice versa, is unsafe.

The inquiry into P metabolism is still centered about the question of the availability of inorganic forms of P for the animal organism. Determinations of the P and N exchange usually indicate better retention when the P is supplied in organic combination (Masslow(*a*); LeClerc and Cook; Hirschler and Terray) and this is likewise the case for Ca retention, but in work on cows it has recently been shown that if the ingestion of a Ca rich food, as hay, is alternated daily with the ingestion of a food low in Ca and to which inorganic phosphates have been added, there is good retention of both P and Ca (Meigs, Blatherwick and Cary). Berg in a metabolism experiment on himself could show no P retention on addition of $Ca(H_2PO_4)_2$ or $Ca(H_2PO_2)_2$ to a diet supplying 3.04 g. HPO_4 daily. On the other hand Forbes(*b*) in experiments on swine finds orthophosphates and hypophosphites as satisfactory forms in which to supply P as are nucleic acid, phytin or glycerophosphates. Fingerling found the same for ruminants and ducks. Osborne and Mendel were able to supply practically all of the mineral constituents in the form of inorganic compounds and still get normal growth in rats. Experimental work is somewhat inconclusive because the effort to prepare a diet supplying enough protein and energy with a minimum of P in organic combination may result in an insufficient supply of the animo acids or of the food accessories (vitamins) and nutritive failure follows irrespective of the form of P. That inorganic phosphates are utilized to a degree is unquestionably established, but there is still a lack of quantitative work which would establish the percentage of absorption from each source. That this is different seems clear from the fact that the percentage of the ingested P retained by infants is higher when they are breast-fed (human milk contains about 77 per cent of its P in organic combination) than when fed on cows' milk which contains about 27.9 per cent of its P organically combined (Keller; Schlossmann). Marshall in a review of the subject concludes that inorganic forms are as satisfactory as organic, but others, notably Sherman and Forbes, take the more conservative view and (are willing to) grant an advantage, though possibly not indispensability, to the organic forms.

Of the mineral constituents of the body P is the most universally required, by bone, muscle, gland and nerve; P retention is the rule and in this respect and because its retention is frequently independent of the N balance, Albu-Neuberg compare P with fat. In infants P retention is 0.02-0.03 g. P_2O_5 per kg. body weight per day, in growing children it is 0.027-0.042 g. per kg. (Herbst(*a*)(*b*)), in adolescent boys it is 0.004-0.015 and may be said to be independent of the N balance, although the lowest P retention found, 0.04 g. P_2O_5 per kg., accompanied a negative N balance. The retention of P_2O_5 was twice as great as would have been required by the retained N and Ca for building bone and muscle. Cronheim and Müller(*b*) found a similar retention of P in excess of the amount required by the retained Ca and N and conclude "P rich nerves

and tissues rich in nuclear material must play an important part in the growth of the early years." Insufficient P in the food during growth results in serious underdevelopment of the bones (Schmorl; Masslow(*b*)).

The partition of the excreted P between urine and feces depends largely on the nature of the diet. A meat diet gives rise to high urinary P and a vegetable diet to a large excretion through the intestine. The urinary excretion is normally 2-2.5 g. P_2O_5 as primary and secondary phosphates of the alkali and alkaline earth metals. Intestinal excretion of Ca and P_2O_5 usually run parallel. Phosphaturia, which is characterized by a cloudy urine or one which becomes cloudy on heating, is not always due to increased amounts of phosphates in the urine, but frequently to their insolubility in an alkaline urine, and may result from a vegetable diet or an ingestion of quantities of alkali or following the increased alkalinity (so-called) of the blood during digestion or loss of the acid stomach juices by vomiting or by removal with stomach pump. Pathological phosphaturia follows an increased alkalinity of the blood as a result of disease, or of increased elimination of P and Ca by way of the kidneys because of some interference with the excretory functions of the intestinal membranes (Soetbeer). P is present in the blood in three forms —lipoid, phosphorus, inorganic phosphates and a form soluble in acids but not precipitated by the ordinary phosphate reagents. "Acid soluble P" includes the latter two and is 2-4.5 mg. P (6.4-14 mg. H_3PO_4) per 100 cc. plasma (Feigl(*a*); Greenwald(*f*)) of which 1-3.5 mg. P (3.2-12 mg. H_3PO_4) is in the form of inorganic phosphates (Marriott and Haessler; Denis and Minot(*g*) in normal individuals. The phosphorus concentration in corpuscles is about 7 times as great as in plasma and shows less individual variations (Bloor; Porte). As a result of many analyses using his nephlelometric method Bloor(*g*) gives the following table of average P distribution in the blood of normal men and women:

	Mgs. H_3PO_4 in 100 c.c. Plasma		In 100 c.c. Corpuscles	
	Men	Women	Men	Women
Total	32	36.2	248.	249.
Acid soluble	10.4	12.4	188.	187.
Inorganic	8.7	11.2	18.7	15.7
Lipoid	22.1	24.9	57.	56.6
Other forms	1.72	1.26	172.	167.

Iron

Iron occupies a unique position among the mineral constituents of the body since its presence in hemoglobin endows the blood with oxygen-carrying capacity. The blood of a man is said to contain about three grams of iron. The liver and spleen contain perhaps 0.02 per cent of their

fresh substance; iron is likewise found in bone marrow and in muscles. As a constituent of nucleoproteins iron has the function of a catalyst (Spitzer) particularly of oxidations, and its presence in most (Mouneyrat; Jones) if not in all cells (Masing) both animal and vegetable has generally been accepted. It has been demonstrated in the liver and other organs of animals whose blood pigment is not hemoglobin (Baldoni; Dastre and Floresco). The cell nuclei of vegetable tissues also contain iron, and the decorticated and enucleated form in which most cereals are used for human food makes them relatively poor purveyors of this element. Some fruits and vegetables, especially the chlorophyll-containing ones, such as spinach and cabbage, are richest in iron. The amount of iron necessary to meet the daily requirements of man cannot be stated dogmatically since it depends on the kind and amount of other foods, organic as well as inorganic, ingested with it (Koehmann(*c*)). In view of our meager knowledge Sherman in his review of the functions of iron in nutrition states that the daily intake ought to be not less than 12 mg. of food iron, a figure which should be increased during pregnancy and lactation. Milk is one of the poorest sources of iron (Jolles and Friedjung; Langstein; Edelstein and v. Czonka). The relative amount of iron in the body of an animal varies with its age; thus Meyer(*a*) showed that in calves the iron of the liver decreases with increasing age; he found that the fetus contained ten times as much iron (relatively) as the grown animal, most of which is accumulated during the last three months before birth (Hugounenq). This question was especially dealt with by Bunge(*b*) and Abderhalden (*e*)(*a*)(*g*), who found, in rabbits and in rats, that the relative amounts of iron and hemoglobin in the body decreased progressively during lactation, at the end of which it was at a minimum. Thereafter, on the mixed food of the mother the iron again increased. In guinea pigs whose lactation period is extremely short, this relation was not observed. Abderhalden therefore points out the undesirability of restricting an infant to milk diet beyond the period of lactation, and the necessity of abundant iron-containing foods for growth and increasing blood volume.

In iron-containing foods the element is usually in complex organic combination; only in drinking water and in medicinal iron preparations is iron ingested in inorganic form. The course which iron follows in the digestive tract has been of special interest because of a possible difference in behavior between the two forms, and in contradiction to the first pronouncements of Bunge(*a*) on the toxicity of inorganic iron and the good fortune of its non-absorption there has come a general acceptance of the view that both forms are absorbed in the same way. The toxicity of iron salts given intravenously was demonstrated long ago, but since inorganic iron *per os* has no toxic effects unless the doses are large enough to erode the epithelium, iron salts are in some way modified in the stomach (Gaule). A part of the ingested iron, either organic or medicinal, is set

free (Schirokauer) forming a loose combination with peptone, perhaps of the nature of an albuminate. Hemoglobin, nucleic acids, and related compounds, on the other hand, are probably not decomposed until after they have left the stomach.

The further course of iron has been followed histologically in the intestinal tract and in organs and tissues by means of a microchemical test with ammonium sulphid (and heat), sometimes with the addition of potassium ferrocyanid and HCl; only the loosely combined iron responds readily to this test, the "organic" iron only after long standing under ammonium sulphid or not at all (Quincke; Matzner). While the mechanism of absorption has not been completely outlined it appears that most of the iron enters the system in the duodenum, either in soluble form in the plasma or through the phagocytic action of leucocytes. In dogs provided with various intestinal fistulas it was observed (Rabe) that 87 per cent of the ingested (inorganic) iron was absorbed before reaching the ileum and a large percentage in the duodenum; but such a study of the absorption of iron is complicated by the fact that iron is also largely excreted by the intestine; this was shown as early as 1852 by Bidder and Schmidt(*a*). They found it in all stages of fasting and later work on fasting (Lehmann, Müller, *et al.*), as well as the experiments of Forster and Voit(*a*) showed that iron was constantly eliminated by the intestinal tract, whether iron-containing food was ingested or not. The length of time elapsing between the ingestion of a given amount of iron and its gradual elimination extending over a period of days or even weeks (Gottlieb(*a*), Hamburger), clearly indicated its absorption and also its excretion. Direct experiments on isolated loops of the intestine were even more final in this regard (Kobert and Koch; Honigmann).

The fact that iron in process of excretion cannot be demonstrated microchemically—the reaction is never obtained in fasting animals (Tartakowsky(*a*)) and disappears in guinea pigs after 24 hours of fasting (Swirski)—suggests that all the iron demonstrable by this test is on its way to absorption. This reaction is regularly obtained in the duodenal epithelium and in the submucosa of the ascending colon; it is seldom obtained in the gastric mucosa (Hochhaus and Quincke; Hari(*a*)) or in the lower small intestine except in cases of abundant iron feeding (Macallum(*a*)) or delayed absorption (Cloetta). Nor was Abderhalden able to find any essential difference in manner of absorption between organic and inorganic iron in animals on a vegetable or meat diet and a more recent investigation by means of the microchemical method (Hueck) has confirmed these statements. Because of the gradual elimination of iron the usual balance experiment of short duration (Stockman and Greig) no matter how accurate, cannot afford far-reaching data on the metabolism of iron.

The intestinal elimination of iron takes place through the epithelium

of the colon, perhaps in very small part by way of the bile. That bile may contain iron has long been known, but the figures given show a wide variation which may be ascribed in part to faulty methods of analysis, in part perhaps to a different behavior of various forms of iron (Leone). The clear connection between hemoglobin and the bile pigments and the place of formation of the latter, unquestionably the liver, need not be reviewed here. The iron thus set free is deposited in the organs or gradually eliminated, but whether the amount of urobilin in the feces is a reliable index of blood destruction in health and in disease is uncertain (Mc Crudden(*d*); Robertson(*a*); Whipple and Hooper(*a*)). Bunge's theory of a protective action of iron salts against hydrogen sulphid in the intestine has been discarded because of the proven absence of hydrogen sulphid in the small intestine (Macfayden, Nencki and Sieber).

The urinary elimination of iron has been the subject of many investigations with widely different results (earlier literature cited by Socin) but by the method of Neumann which gave constant results it appeared to be about 1 mg. in 24 hours, perhaps much less (Marriott and Wolf), a small fraction of which is decomposable by $(NH_4)_2S$ and heat, the rest being in complex organic combination, perhaps of the nature of a pigment or of a non-coagulable protein compound (Monier). A small proportion of intravenously or subcutaneously injected iron appears in the urine (Damaskin), most of it, however, is eliminated by way of the intestine (Lipski). The urinary excretion of iron varies in some pathological conditions (the literature is cited by Goodman), but the kidneys play a minor part in the excretion of iron (Fini; Lapicque; Woltering).

Experiments on iron metabolism date back as far as 1849 when Verdeil showed that the ash of dogs fed meat contained more iron than that of dogs given bread (for the early literature see Hall); the accumulation of iron in the liver after intravenous injection (Zaleski; Gottlieb(*a*)) and after ingestion in organic or inorganic form (Kunkel; Salkowski(*c*); Tartakowsky(*b*); Oerum(*a*); Bonanni(*b*)) especially after the organic (Samoljoff) not only in liver but also in spleen, muscles and bones has been determined repeatedly. The iron-free feeding experiments of v. Hoesslin are the earliest of their kind. By such food and by bleeding he deprived growing dogs of iron; their hemoglobin fell and anemia was also evident in a paleness of the mucous membranes, but growth was not interfered with; similar results were obtained on rabbits. The interesting experiments of Schmidt on mice showed that iron-poor food did not produce anemia or a fall in hemoglobin in full-grown animals but that the offspring of such animals, on the same iron-free food, were retarded in growth and developed severe anemia, with disappearance of iron stores in the liver and their diminution in the spleen. According to Fetzer the administration of iron-poor food to pregnant rabbits and guinea pigs caused a depletion of the iron supplies of the mother up to a certain point, but the maternal

organism did not sacrifice the iron required for its own vital functions. After blood deprivation it appeared (Eger; Häusserman(*a*)) that animals returned to normal hemoglobin slowly on inorganic iron, more quickly on food rich in iron, and most quickly on both. The conclusion of Abderhalden that the addition of iron preparations to food rich in iron is more stimulating to the hemopoietic organs than when it is added to iron-poor food, was not universally accepted; an interesting debate ensued between Abderhalden on the one hand and Jaquet and Tartakowsky on the other, a summary of which is given in Meinertz' excellent review of iron metabolism. From Abderhalden's own figures Tartakowsky showed that the differences in hemoglobin produced by adding inorganic iron to iron-rich and to iron-poor diets were very small, and when taken absolutely were rather in favor of the iron-poor diet with the accompanying relatively smaller total amount of hemoglobin. From histological studies on bone marrow of dogs that had been bled, Hoffmann concluded that the stimulating effect of iron was in speeding up the development of red cells, and Müller(*b*) indeed found more nuclear erythrocytes in the bone marrow of iron-fed animals, but not, he concluded, as a result of stimulation (similar to that of arsenic, perhaps) but simply because of the presence of more raw material. Tartakowsky was able to show that the feeding of iron preparations to anemic dogs on iron-poor food prevented a fall in hemoglobin; iron was still present in liver and spleen two months after beginning the iron-poor food, and he maintained that the blood of full-grown dogs cannot be deprived of iron by feeding iron-poor food. Only bleeding accomplished this and hemoglobin was brought back to normal on iron-poor food by the addition of iron, but not without it. Lack of material is the whole explanation and bleeding in itself is the stimulus. Later results reported by Oerum indicated a distinct superiority of organic iron over the inorganic in restoring loss of hemoglobin although the iron content of liver was greatest in the inorganic iron animals. Zahn on the other hand reports findings indicating that in animals (made anemic by bleeding) hemoglobin did not increase any more rapidly with than without medicinal iron addition to the food. He fed iron-rich food to both groups and this he considers the important difference between his own and previous experiments; perhaps other dietary factors are also involved (Hooper and Whipple(*b*)). Chistoni(*b*) found that organic iron preparations possessed a superiority over inorganic when given intravenously to dogs with experimental anemia; hemoglobin and erythrocytes increased less rapidly with inorganic, and the other pathological indications did not disappear under inorganic iron administration as they did under the organic. More recently the value of inorganic iron in the treatment of secondary anemia has been questioned because Blaud's pills were found to be inert when added to various diets whether these favored blood regeneration or not.

Hemoglobin, on the other hand, exerted a distinctly favorable influence (Hooper, Robscheit and Whipple).

V. Noorden points out that artificially produced anemia is not comparable with chlorosis, nor are the conclusions from experimental results interchangeable, because in this disease it is not a matter of lack of food iron, and the stimulus required by the blood-forming organs seems to be more powerful in inorganic iron preparations than in iron-containing proteins. Evidently no general conclusions can as yet be drawn. From the standpoint of the physiology of nutrition the whole question is, according to Albu and Neuberg, of minor importance since the iron of foods is almost entirely in organic combination. Sherman voices the opposite opinion and considers that it is of great importance to know whether the iron in natural waters can supplement an inadequate supply of food iron. To what extent the full-grown organism can husband its resources of iron is still uncertain but there is no question as to the need of abundant iron in growth and in pregnancy. The retention of iron observed at high altitudes and considered as evidence of the need of additional iron supplies (v. Wendt(*b*)) requires confirmation (Sundstroem(*b*)).

The rôle of the spleen in iron metabolism is uncertain and many of the conclusions reached are quite contradictory. The iron content of the spleen is decreased by repeated bleeding and during pregnancy, and is increased by hemolytic processes and by the administration of iron. Investigations on splenectomized animals indicated that the fecal iron in such animals was considerably above normal (Asher and Grossenbacher; Chevallier(*c*); Bayer(*a*)), especially when loss of body protein was caused by underfeeding, and remained so for many months (Asher and Zimmermann), though these findings have recently not been corroborated (Austin and Pearce). There was some loss of hemoglobin (Pugliese) or none at all unless the food was poor in iron (Tedeschi; Asher and Vogel). Such anemia in dogs was more marked on a diet of cooked meat than when the meat was fed raw (Pearce, Austin and Pepper). Examination of different organs and tissues microchemically and analytically indicated a changed distribution of iron, the liver of guinea pigs containing less than normal (Pana) although an increase is also reported; in frogs a decrease was observed in all tissues and organs (Gambarati). The various changes develop gradually, persist for several months, and finally diminish (Chevallier(*a*)(*b*)) as if other organs developed a vicarious activity. It would seem that the spleen is an organ for the assimilation of iron, and is not necessary for the process of blood destruction (Meinertz(*a*)), but that it retains for the body the iron that has been set free; but whether it does this for the iron resulting from the destruction of erythrocytes (Bayer(*c*)) or for that originating in food is not determined. In cases of pernicious anemia and hemolytic icterus splenectomy has been of advantage; in these cases, however, a previously abnormally large loss of iron in the feces was

very greatly reduced (Goldschmidt, Pepper and Pearce; Pepper and Austin), a result directly opposite to that obtained in normal animals. In experimental anemia the store of iron in the liver and spleen increases (Muir and Dunn), but some factor other than blood destruction is operative, perhaps a derangement of the mechanism for retaining iron (Dubin and Pearce(*a*)).

Sulphur

In a discussion of mineral metabolism sulphur requires only a passing mention, for the amounts of this element ingested in inorganic form are very small. The various forms of sulphur found in the urine (inorganic and ethereal sulphates, neutral and basic sulphur), and in the feces (sulphids) originate in the processes of digestion and utilization of the sulphur-containing proteins in the food and from the catabolism of sulphur-containing tissue proteins. Since sulphates are thus always available in the body it is obviously impossible to determine the requirements of the organism for inorganic sulphur. That the organic form is necessary is indicated by the experiments of Osborne and Mendel(*g*). It appeared that cystin was a limiting factor in growth of rats on a diet containing 9 per cent of casein, since the addition of cystin without any other modification made the ration decidedly more adequate. The addition of cystin to diets low in protein, Lewis(*a*) found, diminished the elimination of nitrogen in dogs while the equivalent amount of nitrogen in sulphur-free compounds such as tyrosin and glycocoll had no such effect. It has recently been shown that rats cannot use inorganic sulphates in place of the necessary amino-acid cystin (Daniels and Rich).

Iodin

Iodin was discovered in the thyroid by Baumann in 1895 in amounts from 2 to 7 mg., in the normal gland; much higher values (3-44 mg.) have been reported recently by Zunz whose data were obtained during the war, and the literature contains widely divergent figures. It is present in the thyroid of cattle long before birth, the female containing more than the male, and it is present in the new-born infant and in the human fetus at least during the last three months of intrauterine life (Fenger(*a*) (*b*) (*d*); Pellegrini). The amount of iodin gradually increases with age, being most abundant at about the age of 50. There is also a seasonal variation in the iodin content of the thyroid (in cattle, sheep and hogs); in the summer and fall the amount of iodin is considerably greater than in winter and spring (Seidell and Fenger; Fenger(*e*)), and is to be associated with external temperature and change in the size of the gland. In cattle no difference was found between pregnant and nonpregnant animals. The iodin content of the thyroid may also be increased by increas-

ing the iodin content of the food and is probably closely dependent upon it normally (Hunter and Simpson; Strauss; Cameron(*a*)). (For a discussion of iodin in foods see Forbes and Beegle; for its distribution in plant and animal tissues see Cameron.) Its absence in the pituitary has very recently been confirmed (Seaman) as well as its presence in the blood (Kendall and Richardson). The complex organic combination in which iodin is found in the thyroid has been isolated and identified by Kendall as 4, 5, 6 tri-hydro-4, 5, 6, tri-iodo-2-oxy,-beta indol-propionic acid, containing 65 per cent iodin and to which most if not all of the physiological effects of the thyroid gland can be ascribed, particularly the stimulation of basal metabolism (Kendall(*a*)(*c*)(*d*); Kendall and Richardson; Cameron and Carmichael).

Iodin compounds are absorbed by the intestine and since iodids are sometimes excreted after administration of organic iodin, while ingested iodids may serve to increase the amount of thyroid complex, the body possesses the ability to ionize and also to deionize iodin (Buchholtz; Blum and Grützner). Inorganic iodids are excreted mostly by the kidneys, and the time of their appearance after ingestion may be used as the basis of absorption tests though marked variation in different individuals is reported. Ingested iodin (element) is quickly bound in the blood by protein and the absorption of iodids by the thyroid is very rapid, but the iodin complex is formed more slowly (Sollmann(*b*); Marine and Rogoff(*a*)(*c*)). The administration of various forms of iodin (non-toxic dose) has caused temporary infertility in animals (Adler(*a*)(*b*); Loeb and Zöppritz).

Lack of iodin in food and drinking water is considered the cause of fetal and maternal athyrosis and as the result of successful treatment in animals the administration of potassium iodid has been recommended (Smith; Hart and Steenbock(*b*); Welch). The administration of small amounts of iodid prevents simple goiter in man (Kimball and Marine), and while this condition has been associated with a lack of iodin (Hunziker), a voluminous literature has established no clear connection between endemic goiter and water supplies (Clark and Pierce). The literature upon metabolism in diseases of the thyroid and in thyroid feeding is reviewed by Halverson, Bergeim and Hawk.

Neutrality Regulation

The maintenance of neutrality is one of the functions of the inorganic constituents of the body. The production of acids in the body is continuous, and the oxidation products of carbon, sulphur and phosphorus are neutralized in the body by the alkali metals (to some extent probably by the alkaline earths), by ammonia resulting from protein decomposition and by the proteins (Klein and Moritz; Robertson(*a*)). The elimination of

carbonic acid as such by the lungs does not involve a permanent withdrawal of alkali from the body, and by virtue of the peculiar ability of the kidney only a portion of the alkali used to neutralize phosphoric acid is lost. The inorganic sulphates of the urine, on the other hand, represent a complete loss to the body of the alkalies required in their formation. The presence of bicarbonate and of phosphates in the blood in optimum concentration is the basis for the delicate mechanism of neutrality regulation which Henderson has so fundamentally conceived. Because of this mechanism assisted by the acid-alkali exchanges between the plasma and the erythrocytes as well as the tissues (Collip; Haggard and Henderson (*b*); Henderson and Haggard), an overproduction of acid, even though it is considerable, does not change the hydrogen-ion concentration of the blood (Sonne and Jarlov); the alkali reserve, as measured by the carbon dioxid capacity, is decreased (Van Slyke and Cullen) and urinary acidity and ammonia are increased. The character of the food influences these relations, foods high in protein and, therefore, containing a preponderance of acid-forming elements decrease the alkali reserve and increase urinary acidity and ammonia, those containing a preponderance of base-forming elements (vegetables, fruits), decrease the latter two and increase the former (Kastle; Forbes(*a*); Sherman and Gettler; Hasselbalch; Blatherwick; McClendon, *et al.*).

Prolonged administration of acids or of acid-forming foods tends to deprive the organism of alkalies. Thus acidosis produced in children by an acid-forming diet caused a loss of Ca and Mg (Sawyer, Baumann and Stevens), and in observations on animals with experimental acidosis the alkaline phosphates, especially the potassium phosphate of the muscles, and the calcium carbonate of the bones were the first major reserves drawn upon after the bicarbonates of body fluids (Goto(*c*)). McCollum(*a*)(*f*) found that rats could grow and be maintained for fairly long periods on acid-forming and also on base-forming rations though reproduction was usually not successful. Lamb and Evvard determined that the addition of sulphuric acid to the ration of swine did not interfere with growth but prevented reproduction. Of the ingested sulphuric acid only 61 per cent was neutralized by ammonia, and their conclusion (that there was no marked loss of calcium) is, according to Forbes, not justified. To what extent these reserves are called into action in daily dietary fluctuations in man cannot be stated; in the experiment of Sherman and Gettler the substitution of isodynamic quantities of rice in place of potato in an otherwise constant diet caused an increase in urinary acidity and ammonia, but the combined increase in both could account for only about two thirds of the acid involved. They suggest that most of the excess might be accounted for by a change in the balance of acid and base-forming substances in the feces, but unfortunately they were unable to make a complete study of the feces. It is significant that

the increased acidity was not accompanied by an increase in urinary phosphorus.

The administration of alkalies to man depresses urinary ammonia and the urine may be made alkaline like that of herbivora (Janney(*a*) Henderson and Palmer); the complete suppression of ammonia cannot be secured in normal subjects though it is possible in nephritis (Denis and Minot(*b*)). The benefit resulting from the giving of alkali in a number of pathological conditions in which acidosis exists, such as diabetes, infantile diarrhea (Howland and Marriott), cholera (Rogers), is temporary and the value of the practice is questioned, but a critical loss of alkali from the blood and tissues is thereby prevented. The acidosis of nephritis (Palmer and Henderson) accompanied by decreased NH_3 excretion is a result not of overproduction but of kidney insufficiency and a consequent retention of acid phosphate; this may even be increased by giving sodium bicarbonate. For this reason the value of Ca in this condition is emphasized (Marriott and Howland(*a*)) because Ca leaves the body largely by way of the intestine; the value of lime in correcting the acidosis of diabetes has also been indicated (Kahn and Kahn(*a*)). The influence of alkalies on the course of sugar utilization and on lactic acid formation, and the effects of acids on nitrogen metabolism, may be cited as further instances out of many others indicating a regulation of the processes of metabolism by the alkaline reserve of the blood and tissues (Underhill(*i*); Murlin and Craver; MacLeod and Fulk; McCollum and Hoagland(*a*); Steenbock, Nelson and Hart).

The important rôle of ionic substances in life processes, in the behavior of the individual cell and in the activity of various isolated tissues, such as nerves, muscles, and especially the heart, need not be considered in a discussion of the metabolism of mineral matter. For normal discharge of its functions every tissue seems to require a properly balanced adjustment of ions in its fluids and membranes and the source of these mineral substances is the ingested food; but to what extent the processes involving ion interactions consume the minerals involved and thereby require their constant renewal in the food, and where the accumulated body reserves are stored, and by what mechanism the physiologically proper proportions of the various ions are selected by the tissues from the heterogeneous supply brought to them by the blood and lymph, are unanswered questions. The tetany following parathyroidectomy may be an example of the unbalancing of ionic equilibrium necessary for normal muscular or nervous activity. Decreased blood calcium accompanies the tetany and administration of calcium relieves it; but the calcium reserves of bone seem not to be available for this purpose. To calcium has been ascribed a very important rôle in correcting almost all kinds of disturbances in inorganic equilibrium, but the translation of inorganic equilibrium into the language of inorganic metabolism must await more knowledge of the terms under which the processes of each are carried on.

Disturbances in Mineral Metabolism Accompanying Pathological Conditions

Fevers are usually accompanied by a retention of chlorids. Snapper (*a*) (*c*) and Peabody have shown that the blood chlorids are below normal, and the retention is due not to a failure of kidney function but to a change in cell permeability. A similar retention of chlorids in fever produced artificially by injection of B. pyocyaneus in dogs has been noticed (Grünbaum). Such chlorid retention is not always accompanied by water retention (Leva(*b*)).

Tuberculosis is accompanied by an abnormal loss of calcium (Croftan; Voorhoeve(*b*); Sarvonat and Rebattu).

Typical hereditary hemophilia is not associated with deficiency in blood Ca, or with irregular Ca metabolism but there is a type of hemophilia "calcipriva" in which the blood calcium is low and in which an increased Ca intake changes a negative to a positive balance with beneficial effects on the blood coagulability (Hess).

Leprosy seems to be associated with a disturbance in Ca metabolism (Underhill(*p*)).

The kidney insufficiency in some types of nephritis is marked by retention of chlorids (Gluzinski; Ceconi).

In nephritis without acidosis the inorganic phosphate of the blood is normal, but with acidosis it may rise to 8-23 mg. per 100 c.c. (Denis and Minot(*g*); Marriott and Howland(*a*)), due to a specific disturbance of kidney function which prevents the elimination of phosphates; at the same time there is a marked reduction of blood calcium. Ingestion of calcium salts, thus diverting the excretory function to the intestine, is recommended as a therapeutic measure.

Attempts to prove an interdependence of mineral metabolism and the endocrin glands have not thus far produced proof of any very definite relationships (Dröge) with the exception of a well-established connection between the parathyroid and Ca metabolism. Underhill, and McCallum with Voegtlin and with Vogel found that the tetany resulting from thyreoparathyroidectomy was accompanied by decreased calcium in the blood and that injection of Ca lactate would temporarily abolish the tetany. "Numerous researches have shown the important relation of the Ca salts to the excitability of the central nervous system. Their withdrawal leaves the nerves in a state of hyperexcitability and tetany may be regarded as an expression of hyperexcitability of the nerve cells from some such cause. The mechanism of the parathyroid action is not determined, but the result, the impoverishment of the tissues with respect to calcium and consequent tetany, is proven." Injections of Ca or Mg salts check the

symptoms of tetany, injection of neutral or alkaline salts of Na or K intensifies them.

By intravenous injection of phosphoric acid and its Na salts Binger has been able to reduce the Ca of the serum from 10 mg. to 6 mg. per 100 c.c. Tetany results at this point unless the pH is above 6; if the solution injected has a pH greater than this no tetany results. A similar marked reduction of blood Ca to as low as 1.5 mg. per 100 c.c. without tetany occurs in nephritis where the blood is extremely high in acid phosphates. Parathyroidectomy is accompanied by an increase in the acid phosphates of the blood and during a tetanic seizure the ammonia of the blood is about twice normal, while injection of ammonium carbonate into normal animals will bring on symptoms of tetany immediately (Greenwald(*a*)(*b*); Watanabe(*c*); Jacobson). That the hydrogen ion concentration is a determining factor is clear from the work of Binger and of Marriott and Howland, and from recent work which showed increased alkalinity of the blood following parathyroidectomy and just before convulsions began (Wilson, Stearns and Thurlow); also from the coincidence of tetany and increased alkalinity of the blood as a result of intravenous infusion of $NaHCO_3$ (Harrop(*a*)), and of operations on the stomach which exclude the acid secretion from the duodenum (McCann). On the other hand, blood which has been dialyzed against a solution containing everything normal to blood except calcium when transfused into the isolated leg of a dog resulted in over-stimulation of the nerves (MacCallum, Lambert and Vogel).

There is some difference of opinion regarding the blood Ca in infantile tetany, Longo (quoted by Howland and Marriott), finding a normal content in eight cases while others have found it much reduced (Neurath; Brown, MacLachlan and Simpson), and Howland and Marriott say "convulsions may be expected when the Ca of the serum becomes less than 7 mg. per 100 c.c." They find the Mg and inorganic phosphates of the blood remain normal. Calcium absorption is little if at all affected in infantile tetany (Schwarz and Bass) and while the Ca content of nervous tissue has been found (post mortem) below normal (Quest; Weigert(*b*)) it is not invariably so; but in cases where the Ca is normal the Na and K are abnormally high, and the ratio $\frac{Na + K}{Ca + Mg}$ is high(Aschenheim(a)). A metabolism study of a baby having rickets and tetany(Fletcher) has brought out a similar relation in the retention of these elements; while the disease was in active progress the retention of CaO was 0.39 gr. daily and the ratio $\frac{Na + K}{Ca + Mg} = 1.5$, during the later period during which there was marked improvement in the symptoms the retention of CaO was 0.44 gr. daily, and the ratio $\frac{Na + K}{Ca + Mg} = 0.72$. Howland and Marriott

have not been able to show alkalosis in cases of infantile tetany, but medication with $NaHCO_3$ for other causes has in four cases resulted in tetany convulsions accompanied by low blood Ca, both of which were corrected when the $NaHCO_3$ was stopped. They conclude "it is apparent that the symptoms of tetany and the lowering of the Ca content of the serum may be produced in a variety of ways, but we have not been able to show that any of these means is operative in infantile tetany."

Administration of Ca salts *per os* may or may not (Haskins and Gerstenberger) have a beneficial effect on infantile tetany. Injection of calcium lactate gives temporary relief and if accompanied by administration of phosphorized cod liver oil it speeds the recovery which phosphorized cod liver oil alone will accomplish (Brown, *et al.*).

There is apparently an intimate relation between blood sugar and calcium. Thyreoparathyroidectomy is accompanied by a decrease in both, and the injection of Ca will temporarily restore blood sugar to normal (Underhill(*h*); Underhill and Blatherwick). The question as to whether the hypoglycemia is a result of the thyreoparathyroidectomy or of the resulting reduction in blood calcium is still unanswered. Hyperglycemia occurs in pneumonia, tuberculosis and especially diabetes, and each of these diseases is characterized by loss of calcium (Kahn and Kahn; Loeper and Bechamp) and upon injection of calcium salts the glycosuria is decreased. Administration of $CaCl_2$ to diabetics is claimed to reduce the glycosuria (Phocas). Urinary elimination of phosphorus is about normal, that of Ca and especially of Mg is high in diabetes (Euler and Svanberg; Nelson). In experimental diabetes in rabbits a decalcification has been observed (Robert and Parisot). There is possibly some connection between the diabetes of pregnancy and the unusual drain on calcium (Kahn and Kahn (*a*)). In the acidosis of diabetes the loss of Ca may be due to the elimination through the urine of Ca salts of volatile fatty acids (Palacios).

Because of the marked changes in mineral metabolism and in the composition of the bone in rachitis and osteomalacia (Goldthwaite, *et al.*; Holt, Courtney and Fales(*d*)(*e*); Schabad(*a*)(*b*); Schloss(*b*); Brubacker; McCrudden(*a*)(*c*)) these have often been considered diseases of lime metabolism. There is usually a negative lime balance in the active stage of rachitis, but rachitis does not always result from a low Ca intake and it frequently occurs in children receiving plenty of CaO. The blood Ca is not invariably abnormal in rickets or osteomalacia (Stheeman and Arntzenius). Attempts to establish a relation between the thyroid, thymus, or sex glands and rickets or osteomalacia are not convincing (Sarvonat and Roubier; Zuntz(*c*); Bieling; Claude and Rouillard; Rominger; Aschenheim(*c*)). The seasonal variation of rachitis, its incidence being greatest in the spring and least in the early fall months, has been associated with the increased Ca retention shown by lactating cows when changed from a dry to a fresh green ration containing the same amount of Ca.

Possibly the lack of some food accessory which affects mineral metabolism (as for example the antiscorbutic vitamin) and which is reduced by drying, is reflected in the milk and results in the appearance of a pathological condition in a young animal subsisting on that milk (Baumann and Howard; Hart, Steenbock and Hoppert; Robb).

The disturbance of phosphorus metabolism accompanying that of calcium metabolism in rachitis has been considered a secondary effect. The fact that phosphorus therapy is frequently successful (Kochmann(*d*); Meyer(*b*)) suggests that phosphorus may be more fundamentally involved than it is generally thought to be.

Osteomalacia, on the other hand, is more generally considered a disease of calcium metabolism, occurring usually as a result of the drain on body lime during pregnancy. McCrudden(*c*) considers that the normal "flux" of calcium is increased in pregnancy, that because of functional inertia it may continue too long after the demand has ceased, and become pathological, and that ovariotomy effects a cure, not because of any functional relation between the ovaries and Ca metabolism, but because it removes the possibility of further drain on calcium by pregnancies. The effect of castration on rats bears this out since the lime content of females is unchanged by castration, but that of males is reduced 10-20 per cent (Reach).

The Metabolism of Vitamins. . . . . *Carl Voegtlin*

Discovery of Vitamins—Chemical Nature and Physical Properties of Vitamins—Antineuritic Vitamin (Water-soluble B)—Fat-soluble Vitamin (Fat-soluble A)—Antiscorbutic Vitamin (C Factor)—Distribution of Vitamins in Food—Digestion and Absorption of Vitamins—Intermediary Metabolism and Physiological Action—End Metabolism of Vitamins—Special Feature of Vitamin Metabolism.

The Metabolism of Vitamins

CARL VOEGTLIN

WASHINGTON

Discovery of Vitamins

Until a few years ago it was generally assumed that a complete diet for purposes of proper growth and maintenance of health of the animal body should consist of proteins, fats, carbohydrates, inorganic salts and water in sufficient quantities to furnish an adequate supply of energy and material for the building up of the body tissues. The discovery of certain other substances not related to the above-mentioned food factors, and now considered just as essential for the maintenance of normal metabolism, can be traced back to two distinct lines of investigation; first, the study of scurvy and beriberi, and, second, feeding experiments with highly purified diets.

Numerous clinical observations on scurvy and beriberi, and especially the experimental production of these diseases in the lower animals by Eijkman(*c*) (1897), and Holst and Fröhlich(*a*) (1907), called attention to the importance of the diet in the causation of these diseases. Thus it was found that scurvy does not occur if the diet contains an adequate amount of either fresh meat, fresh vegetables or fresh fruits, and that the disease can be successfully treated by the administration of *relatively small* amounts of certain fresh fruits and vegetables. These observations, and the fact that prolonged exposure of these foods to temperatures of 100° C. destroyed their prophylactic and curative properties, suggested that the fresh foods contained some hitherto unrecognized food constituents. Experience with beriberi showed furthermore than this disease appears if the diet is restricted to highly milled cereals, whereas people living on foods made from the whole grain are immune against beriberi. Small amounts of an extract of the portion of the grain removed in the milling process proved to be a powerful curative agent. This led to the conclusion that the whole grain and the extracts made from the offal contained a substance or substances which later on were shown by Funk(*a*) (1911) not to be related to any of the well-known food factors.

Independent of this work on scurvy and beriberi, some investigators attempted to feed animals on purified diets containing an adequate pro-

portion of the well-known food factors (purified proteins, fats, carbohydrates and inorganic salts). These attempts invariably resulted in failure, as the animals after a certain period declined in weight and exhibited symptoms of malnutrition. Pioneer work on this subject was done by Lunin (1881), Stepp(*a*)(*b*) (1909, 1912), Hopkins(*a*) (1912), Osborne and Mendel (1911), and McCollum and Davis(*d*) (1912, 1915).[1] The addition of small quantities of milk or certain other natural foods to the purified diet rendered the latter physiologically complete. The purified diet, as the diet which causes beriberi or scurvy, was evidently lacking in some food constituents which are essential for normal metabolism. These substances of unknown chemical composition were termed by Funk "vitamins." Hopkins refers to them as "accessory food factors," and McCollum speaks of the "Fat-soluble A" (fat-soluble vitamin), "Water-soluble B" (antineuritic vitamin), to which Drummond has added the "Water-soluble C" (antiscorbutic vitamin).

There can be little doubt, if any, about the identity of the antineuritic vitamin with the water-soluble B. The proof for this assumption is based upon two well established facts: (1) the solubilities in various solvents and the resistance towards heat, exposure to alkali and other agents is identical for both substances; and (2) the distribution of these two factors in various foodstuffs is the same, whether established by means of growth experiments on rats or whether the antineuritic power is determined in pigeons. Both pigeons and rats develop polyneuritis if the diet is lacking in either water-soluble B or antineuritic vitamin.

All the various terms applied to these substances have been justly criticized for one reason or another. The terminology adopted in this chapter should therefore be considered as more or less arbitrary.

Chemical Nature and Physical Properties of Vitamins

The chemical composition of vitamins is unknown, principally on account of past failures to isolate these substances in pure form from the natural foods. The work so far done on this subject is, however, not without interest, both from a theoretical and practical aspect, and will therefore be briefly reviewed.

Antineuritic Vitamin (Water-soluble B).—The early researches on beriberi and polyneuritis gallinarum showed that the antineuritic vitamin can be readily extracted by means of water or hot ethyl alcohol (Eijkman (*e*), 1906) from rice polishings, yeast, and other material rich in this substance. Acetone, ether, chloroform, benzene, and petrolether fail to ex-

[1] For a historical review of the earlier experiments, the reader is referred to the monograph by Osborne and Mendel (1911). The later development of the subject is admirably presented in the "Report on the Present State of Knowledge Concerning Accessory Food Factors (Vitamins)," Medical Research Committee, 1919, H. M. Stationery Office, Imperial House, Kingsway, London, W. C. 2.

tract this vitamin (McCollum and Simmonds(*a*), 1918). The addition of a small amount of hydrochloric acid to alcohol increases the efficiency of the extraction and the best results are obtained by using acid methylalcohol (Voegtlin and Myers(*d*), 1920). If the alcoholic extract is deposited upon dextrin and the mixture dried, the deposited vitamin may be dissolved by benzene, but not by acetone (McCollum and Simmonds(*a*), 1918). Voegtlin and Myers(*d*) (1920) showed that olive oil or oleic acid extracts the antineuritic vitamin from autolyzed yeast, thus proving that at least under certain conditions this vitamin is fat-soluble, as well as water-soluble. The great water-solubility of this vitamin suggests that in the cooking of fresh foods in water a considerable amount of this substance may pass into the water, and that the latter should therefore be consumed with the cooked food whenever possible. The active substance diffuses easily through the ordinary semi-permeable membranes (Chamberlain and Vedder(*a*)(*b*), 1911, and Sugiura, 1918), a fact which indicates that the antineuritic vitamin very probably has a relatively small molecular weight. It is safe to regard the antineuritic vitamin as it occurs in the natural foods as resistant to drying or moderate heating, up to 100° C. Prolonged heating of foods above 100° C., as used in the process of commercial canning, appears to destroy a variable proportion of this factor (Grijns, 1901; Eijkman(*e*), 1906; Holst, 1907; McCollum and Davis(*d*), 1915). In an alkaline medium destruction proceeds much more rapidly, especially if the temperature is raised to 100° C. (Cooper(*a*), 1913; Vedder and Williams, 1913; Sullivan and Voegtlin(*a*), 1916; Steenbock(*a*), 1917; Drummond(*a*), 1917; Chick and Hume(*d*), 1919). For example, it was found that cornbread made from low extraction cornmeal, baking soda, salt and water was deficient in antineuritic vitamin, whereas cornbread made without the addition of sodium bicarbonate still contained this vitamin (Voegtlin, Sullivan and Myers, 1916). The use of baking soda in cooking is therefore contraindicated unless proper provisions are made to neutralize the free alkali, as for instance by the addition of buttermilk in bread making. Several observers (Cooper and Funk, 1911; Sullivan and Voegtlin(*a*), 1916) have noted that the antineuritic substance is highly resistant to acids, as prolonged boiling with 10 p.c. sulphuric or hydrochloric acid does not seem to lead to any appreciable deterioration; on the contrary, the physiological activity of crude extracts of foods containing this vitamin was greatly increased by this treatment, as shown by the prompt relief of the symptoms in polyneuritic birds (Vedder and Williams, 1913; Sullivan and Voegtlin, 1916).

Zilva (1919) has demonstrated that the antineuritic vitamin in autolyzed yeast is not destroyed when exposed for six hours to ultraviolet rays, nor does radium emanation seem to have any deleterious action upon this substance (Funk(*e*), 1916). Sugiura and Benedict (1919) claim that the growth-promoting factors in yeast may be partially inactivated by this

treatment, an observation which these observers consider as a possible explanation of the therapeutic effect of radium upon neoplasms.

Cooper and Funk (1911) discovered that the active substance is precipitated by phosphotungstic acid, and that the precipitate thus obtained yields a highly active preparation after decomposition of the precipitate and removal of the phosphotungstic acid. Later work by Funk (1912, 1913) then showed that this preparation can be further purified by treatment with silver nitrate and baryta, which precipitates the vitamin. By repeated recrystallization of this fraction (pyrimidin fraction), a substance was obtained which melted at 233° C. to which Funk gave the formula $C_{17}H_{20}O_7N_2$. The crystals, for unknown reasons, very often lose their physiological activity on recrystallization from water, a fact which has been most troublesome in the isolation of this vitamin. The principal observations of Funk were confirmed by Edie, Evans, Moore, Simpson and Webster (1911-12), Cooper(*a*)(*b*) (1913), Vedder and Williams (1916), Williams (1916), Voegtlin and Myers(*d*) (1920), and others. The last two investigators carried the purification a little further by the use of mercuric sulphate, and obtained a product free of purins, histidin, proteins, albumoses and lipoids. Suzuki, Shinamura and Odaki (1912) claim to have prepared a picrate of the antineuritic vitamin, but their work could not be verified by Drummond and Funk (1914). Hofmeister(*a*)(*d*) (1918, 1920) claims that the antineuritic vitamin belongs to the pyrimidin series ($C_5H_{11}NO_2$) and that it yields a crystalline hydrochlorid and gold salt. Williams and Seidell (1916) obtained adenin from autolyzed yeast, and found that it had powerful curative properties when tested on polyneuritic birds. The sample lost its physiological properties on standing. They furthermore found that inactive adenin submitted to treatment with sodium ethylate assumed antineuritic properties, observations which led these authors to regard the antineuritic vitamin as an isomer of adenin. However, Voegtlin and White (1916), and Harden and Zilva(*a*) (1917) were unable to confirm these observations.

The active preparations and crystalline fractions hitherto obtained by various workers are probably mixtures of active material and impurities, and the passing over of the active substance into certain fractions is explained by Drummond(*a*) (1917) by the assumption that this vitamin is easily carried down by bulky precipitates. The antineuritic vitamin is adsorbed by charcoal (Chamberlain and Vedder(*a*)(*b*), 1911), by fullers' earth (Seidell, 1916), by mastic emulsion or basic ferric phosphate (Voegtlin and Myers(*d*), 1920), and by colloidal ferric hydroxid (Harden and Zilva(*c*), 1918). Of these absorbing agents, fullers' earth appears to be the most suitable one for the purpose of preparing a quite stable concentrate from aqueous solutions containing this vitamin. The activated fullers' earth can be made use of as a source of this vitamin in feeding experiments (Eddy, 1916). Adsorbing agents have so far not been of

assistance in the chemical isolation of this vitamin, probably on account of the fact that other material, especially organic bases, are also carried along with the active substance. In any attempt at the isolation of this vitamin, proper consideration should be given to the possible injurious effect of alkali and heat.

Fat-soluble Vitamin (Fat-soluble A).—This dietary factor was first discovered in butter (McCollum and Davis(*a*), 1913; Osborne and Mendel (*c*), 1913), and is usually found in association with certain food fats in which it is very readily soluble. It can be extracted from dried spinach or clover by ether (Osborne and Mendel(*r*), 1920). In water it is only soluble to a very limited degree. McCollum (1917) has estimated, for instance, that in milk one-half of the substance present is dissolved in the milk fat, which indicates that the solubility in fat is approximately 30 times greater than that in water. Osborne and Mendel(*h*)(*q*) (1915, 1920) observed that butter fat treated with live steam for 2½ hours had not lost any of its fat-soluble vitamin. More recently Steenbock, Boutwell and Kent (1918) claimed, however, that the substance is slowly destroyed at 40° to 60° C., and that complete destruction takes place after 4 hours' exposure to 100° C. These observations were confirmed by Drummond (*e*) (1919), who worked with butter and whale oil. The fat-soluble vitamin in plant tissues is not destroyed by autoclaving for three hours at 15 pounds pressure (Steenbock and Gross(*b*), 1920). The destructive process is evidently a reaction of slow velocity, but of sufficient magnitude to be considered from the practical point of view of the deterioration of this factor in food.

Saponification of butter fat with alcoholic sodium hydroxid does not destroy the fat-soluble vitamin (McCollum and Davis(*c*), 1914), whereas saponification in the presence of water leads to complete destruction (Drummond(*f*), 1919). In the commercial "hardening" of certain oils by means of hydrogen, the physiological activity originally present in the oil is lost, this being principally due to the high temperature used in this process (Drummond, 1919). This vitamin is also destroyed when butter is exposed for 8 hours to ultraviolet rays (Zilva, 1919). There is a complete lack of knowledge regarding the chemical composition of this substance, although recently Steenbock (1919, 1920) has called attention to the possible identity of this substance with a yellow pigment, carotin, a view which, however, is not shared by Palmer (1919).

Antiscorbutic Vitamin (C Factor).—This vitamin is soluble in water and alcohol (Harden and Zilva(*b*), 1918; Hess and Unger(*b*), 1918) and is easily dialysable through parchment (Holst and Fröhlich(*b*), 1912) and porcelain filters (Harden and Zilva(*d*), 1918). The substance loses its physiological activity on drying, sometimes even at low temperature, and more readily at 100° C. (Givens and Cohen, 1918; Givens and McCluggage(*b*), 1919). From the experiments of Delf (1918) it appears that the

rate of destruction of the antiscorbutic vitamin contained in fresh cabbage is accelerated about threefold when the temperature is raised from 60° to 100° C. The destructive action of heat is more pronounced when the substance is heated in an alkaline medium (Holst and Fröhlich(*b*), 1912; Hess and Unger(*d*), 1919), whereas an acid or neutral reaction seems to stabilize it somewhat (Harden and Zilva, 1918). The effect of canning on the antiscorbutic factor of vegetables was studied by Campbell and Chick (1919). Nothing is known concerning the chemical composition of the antiscorbutic vitamin.

The principal feature brought out by this brief discussion of the physical properties of vitamins is the fact that these substances must be considered as relatively unstable, because various influences tend to destroy their physiological properties. It is readily seen that this lack of stability has an important bearing upon human nutrition and a proper appreciation of this fact, combined with further work on this subject, will ultimately lead to more rational methods of manufacture and cooking of foods.

Distribution of Vitamins in Food

From the standpoint of practical dietetics, it is of great importance to determine the vitamin content of the more commonly used foodstuffs. The available data bearing on this point were obtained by means of feeding experiments on rats, guinea-pigs, pigeons and chickens. To a basal diet, complete in every respect but lacking the vitamin under consideration, there were added the foodstuffs to be investigated in such amounts as to just maintain normal nutrition or growth (Chick and Hume(*d*), 1919). The results, which of course are not absolutely accurate, may be briefly summarized as follows: The principal sources of the *antineuritic vitamin* are the seeds of plants, eggs, animal tissues, with exception of adipose tissue, the green parts of plants, pulses, and to a more limited extent, milk, fruits, and tubers. Brewers' yeast is very rich in this factor. In the case of cereals, this vitamin is principally, if not exclusively, located within or close to, the embryo, which accounts for the deficiency of the highly milled products in this factor, as the milling process removes the embryo and superficial layers of the seed.

The *fat-soluble vitamin* is largely found associated with certain animal fats, and also occurs in the green parts of plants, and to a lesser extent in the germ of cereals. Butter, cream, fish oils, and egg yolk are rich in this factor, whereas lard, and the vegetable oils do not contain it in appreciable quantities. No explanation is available for the paradoxical fact that beef fat contains the fat-soluble vitamin and that the latter is not present in lard.

The main sources of the *antiscorbutic vitamin* are fresh, green

vegetables, certain fruits, and, to a more limited extent, fresh meat, tubers and fresh milk. In general, dried milk powders (Barnes and Hume, 1919), condensed and pasteurized milk (Hess(*c*), 1916) are deficient in this factor. It is interesting to note that the germination of cereals leads to the formation of the antiscorbutic vitamin, as shown by the action of sprouted grains in the treatment and prevention of scurvy in guinea-pigs (Fürst, 1912; Weill, Mouriquand and Peronnet, 1918; McClendon, Cole, Engestrand, 1919).

An important relationship between the dietary value of the natural foods was brought out by the systematic investigation of McCollum and his coworkers (1917), who were able to show that the addition of the green parts of plants to a diet restricted to the seeds of plants has a marked tendency to render the diet more complete not only with respect to the inorganic salts but also the fat-soluble vitamin; and previous work had shown that green vegetables supply furthermore the antiscorbutic vitamin which is absent in cereals. The conclusion is therefore justified that a proper mixture of the green parts of plants and the seeds does possess a higher dietary value than either of these foodstuffs alone. A mixed diet containing, in addition to cereals and green vegetables, also some milk and fresh meat is the best safeguard against the possibility of a vitamin deficiency and furthermore insures an adequate supply of inorganic salts and protein of proper biologic value.

The table on pages 352-355 includes the principal data regarding the distribution of the three vitamins in the common foodstuffs. The information contained therein may be of sufficient practical value until more accurate methods are worked out for the quantitative estimation of vitamins in foods. The relative quantity of these substances is indicated by the number of plus signs. A zero sign signifies total absence or insignificant traces.

Digestion and Absorption of Vitamins.—In view of the relatively unstable character of vitamins it is a matter of importance to know whether these substances are partly destroyed during digestion. Quantitative information on this point is completely lacking. However, it may be safely assumed that the utilization of the vitamins contained in certain foods (yeast, butter, lemon juice) is fairly efficient, as very small quantities of the latter are required to supply the animal's needs in vitamins. Whether vitamins are absorbed by the stomach or the upper intestines or by both of these organs remains to be determined.

Intermediary Metabolism and Physiological Action

After absorption from the gastrointestinal canal, the vitamins are carried, presumably by way of the portal circulation, or possibly also the lymphatics, to the tissues of the body, where they are stored up. It is

interesting to note that different organs vary considerably in their vitamin content. Thus Cooper(*b*) (1913) has shown that the antineuritic vitamin content is largest in ox liver, less in ox heart, and still less in ox brain and skeletal muscle, the latter containing only relatively small amounts of this substance (see also Osborne and Mendel(*j*)(*k*), 1917, 1918). The presence of this vitamin was also demonstrated in the spinal cord (Voegtlin and Towles, 1913), the pancreas (Eddy, 1916), the kidney (Osborne and Mendel(*j*)(*k*), 1917, 1918), and testicle (Schaumann, 1910); whereas it seems to be absent from adipose tissue generally. These observations are rather significant, as they suggest that the antineuritic vitamin is needed in all tissues, more or less in proportion to the magnitude of their metabolism, but not in tissues which function as a depot for reserve energy. This interpretation is also supported by the fact that the yolk of eggs are rich in this substance, whereas it seems to be absent in egg white. A similar deduction may be drawn from the distribution of this substance in plant tissues, as it was shown that it is concentrated within or in the immediate neighborhood of the embryo or germ of the corn and wheat kernel and that it is absent in the superficial layers and endosperm (Voegtlin and Myers(*b*), 1919). More recent work has also shown that the green parts of plants contain considerable quantities of antineuritic vitamin, when due allowance is made for the high water content of these foods (Osborne and Mendel(*n*), 1919).

A somewhat different situation is met with in the distribution in the body of the fat-soluble vitamin, which is found not only in glandular organs, but also in certain adipose tissue (beef fat). Strange to say, it is absent from lard, and skeletal muscle appears to contain only traces. Again, the liver is relatively rich in this substance, as shown by the high activity of cod liver oil.

Almost no data are available concerning the localization of the antiscorbutic vitamin in the various organs of the body, with exception of the well established fact that fresh lean meat contains this factor.

The numerous feeding experiments with deficient diets permit us to conclude that the animal body, under normal conditions, contains a considerable reserve of fat-soluble vitamin, but not of antineuritic and antiscorbutic vitamin. Thus susceptible animals survive a much longer period when supplied with a diet lacking in the former, than on a diet deficient in the latter two vitamins.

As regards the rôle played by vitamins in metabolism, we are still more or less limited to hypothetical considerations supported to some extent by suggestive observations. One of the most perplexing questions is the fact that different species of animals have different vitamin requirements. For instance, it is well proven that a diet complete in every respect but completely lacking the antiscorbutic vitamin will support normal metabolism, growth and maintenance of health in rats, mice, pigeons and chickens for considerable periods, whereas this same diet will cause scurvy

within a few weeks in man, guinea-pigs, monkeys and dogs. On the other hand, it has been conclusively shown that all of the higher animals need a certain amount of fat-soluble and antineuritic vitamin for proper nutrition, maintenance of normal growth, reproduction and life. It has been suggested by various students of this subject that the antineuritic vitamin is somehow concerned with the maintenance of the proper function of the nervous system, an assumption which is supported by the occurrence of severe paralytic symptoms and degenerative changes in the nervous system of animals fed on a diet deficient in this vitamin. More recently, McGarrison has shown, however, that the nervous system is by no means the only organ affected by this particular vitamin deficiency.[2] A few workers have made the attempt to prove that the antineuritic vitamin has a stimulating action upon various digestive glands, this resulting in an increased production of secretion. Bickel(*e*) (1917), for instance, showed that a crude extract of spinach contains a principle with a pharmacological action similar to that of pilocarpin. Uhlmann(*a*)(*b*) (1918) studied the effect of the residue of an alcoholic extract from rice polishings on various digestive glands and the sweat glands. He obtained a marked increase in secretion, following the parenteral injection of the extract. He was furthermore able to show that the same extract caused contraction of intestinal muscle and a fall in blood pressure. The latter effect he attributes to a direct depressing effect on the heart muscle and to vasodilatation. Shortly after this paper had appeared, Voegtlin and Myers(*c*) (1919) published their findings, which were carried out without a knowledge of Uhlmann's work. They showed that the intravenous injection of a highly purified extract from yeast produced an abundant flow of pancreatic and biliary secretion, resembling in every respect the effect produced by an extract of the duodenal mucosa purified in the same manner as the yeast extract. Alcoholic extracts from liver produced the same effect, and all three extracts were shown to be rich in antineuritic vitamin, when tested as to their therapeutic action on polyneuritic pigeons. As suggestive and interesting as these findings may be, it should be emphasized that the physiological effect noted by all these observers may have been due to some highly active impurity and not the vitamin per se.

Dutcher (1918) has recently suggested some relation between the antineuritic vitamin and oxidative processes, as he observed that the tissues of polyneuritic birds showed a marked reduction in catalase and that the catalase activity was again restored to normal after the administration of this vitamin. He believes that this substance increases the production of catalase.

Funk (1919), Braddon and Cooper (1914) claim that the antineuritic vitamin is essential for the metabolism of carbohydrates, a view which is not shared by Vedder (1918).

[2] For further details see chapter on beriberi.

Drummond(*d*) (1918) studied the metabolism of rats fed on an artificial diet deficient in antineuritic vitamin and noted the presence of creatinuria, accompanied by decrease in food consumption. The addition of the vitamin to the diet was followed by an increased food intake.

Incidentally, reference is made to the work of Mellanby(*c*)(*d*) (1919), who claims to have produced experimental rickets in dogs by means of a diet deficient in fat-soluble vitamin, which would indicate that the substance is concerned in the metabolism of calcium. It is impossible to accept this view without considerable modification, as Hess and Unger(*f*) (1920) have shown conclusively that infants develop rickets while receiving "a full amount of this principle, and that they do not manifest signs, although deprived of this vitamin for many months, at the most vulnerable period of their life." McCollum and Simmonds (1920) have also presented evidence which is not in agreement with Mellanby's views.

A lack of fat-soluble vitamin in the diet leads to the appearance of xerophthalmia in rats (McCollum); a condition which had previously been observed by Mori (1904) in young children whose diet was lacking in certain fats, which are now known to be rich in fat-soluble vitamin.

The antiscorbutic vitamin is probably concerned in the growth of some species, but not of all, as Hess(*c*) (1916) observed the appearance of scurvy in infants in spite of a preceding period of normal growth. Holst and Fröhlich have described great fragility of the bones in guinea-pigs suffering with scurvy which on histological examination was shown to be due to lack of proper calcification. It would thus appear that the antiscorbutic vitamin has some relation, either direct or indirect, to calcification.

To sum up, very little indisputable knowledge is available as to the part played by vitamins in metabolism beyond the fact that the antineuritic and fat-soluble vitamin are needed for growth and that all three vitamins are essential for proper nutrition of man and some of the higher animals. Taking into consideration that apparently very small amounts fulfil the physiological requirements, it is quite possible that vitamins act as catalysts of some metabolic reactions. They may also possess an indirect effect upon nutrition by stimulating the digestive organs in the way indicated above.

End Metabolism of Vitamins

The available evidence regarding the ultimate fate of vitamins in the animal body does not permit many positive conclusions. The only data with a bearing on this point are a few observations on the vitamin content of the various secretions and excreta. Muckenfuss (1918) treated saliva, ox bile and human urine with fullers' earth and fed these samples of fullers' earth to pigeons showing acute symptoms, as a result of a polished rice diet. Improvement was noted when the preparation was given in

amounts corresponding to 950 to 3,250 c.c. of ox bile, 400 to 1,325 c.c. fresh saliva or 4,150 to 6,000 c.c. of urine; from which the author concludes that this vitamin is probably present in comparatively small amounts in saliva, bile and only in traces in urine. Some unpublished experiments by Voegtlin and Myers also indicate that human urine obtained from subjects on a mixed diet is very poor in antineuritic vitamin, as "activated" fullers' earth corresponding to over a liter of fresh urine, when fed daily to pigeons on a polished rice diet, was not capable of delaying the onset of polyneuritis.

Cooper(*c*) (1914) showed that alcoholic extracts of feces of rice-fed hens and bread and cabbage-fed rabbits relieved the symptoms of polyneuritic pigeons. This would indicate that at least part of this vitamin is excreted with the feces. (See also Portier and Randoin, 1920.)

That the mammary gland secretes all three vitamins is well established, as feeding experiments with fresh unheated milk has shown that this food belongs to the richest sources of fat-soluble vitamin and that it contains also some antiscorbutic and antineuritic vitamin, although the last two factors seem to be present in relatively small amounts.

The evidence thus far points to the destruction of vitamins within the body, which renders it necessary to constantly replenish the supply through a proper diet. The ultimate source of this supply is the plant, as the animal tissues are unable to produce vitamins.

Special Features of Vitamin Metabolism

A discussion of the metabolism of vitamins would not be complete without a brief reference to the factors which safeguard an adequate supply of vitamins to the young animal during the period of its life when it is entirely dependent upon the milk of its mother. On the basis of some work on rats, McCollum and Simmonds (1918) conclude that milk varies in nutritive value according to the composition of the food fed the lactating animal. The mammary gland has no power of synthesising vitamins (McCollum, Simmonds and Pitz, 1916). An inadequate supply of fat-soluble and antineuritic vitamin in the diet leads to a corresponding diminution of these substances in the milk. Similar observations were made more recently by Hart, Steenbock and Ellis (1920) with regard to the antiscorbutic vitamin content of milk. They have found that summer pasteur milk is much richer in this factor than dry feed or winter-produced milk. (See also Barnes and Hume, 1919.) Osborne and Mendel(*q*) (1920) have found little if any difference in the antineuritic vitamin content of cows' milk during the various seasons, an observation which is easily explained by the fact that the drying of feed does not destroy this vitamin. Further evidence along this line will be found in the chapter on beriberi.

TABLE OF VITAMINS

Classes of Foodstuff	Fat Soluble Vitamin A	Antineuritic Vitamin B	Antiscorbutic Vitamin C	References
FATS AND OILS				
(*Vegetable*)				
Almond oil	0	0		Osborne and Mendel, 1915; McCollum and Davis, 1915.
Cocoanut oil	0			Halliburton and Drummond, 1917.
Cottonseed oil	0	0	0	" " " "
Margarin prepared from vegetable fats or lard	0			" " " "
Nut butters	+			" " " "
Olive oil	0	0	0	McCollum and Davis, 1914, 1915; Osborne and Mendel, 1915.
(*Animal*)				
Beef fat	++			Osborne and Mendel, 1915.
Butter	+++	0		Osborne and Mendel, 1914; McCollum and Davis, 1913.
Cod liver oil	+++	0	0	Osborne and Mendel, 1914; Hess and Unger, 1918.
Cream	++	0		Drummond, 1918.
Fish body oil	++	—	—	" "
Fish fat	+	+	+	" "
Lard	0	0	0	Osborne and Mendel, 1915; McCollum and Davis, 1915; Sullivan and Voegtlin, 1916.
Margarin prepared from animal fat	Value in prop. to amt. animal fat contained			Halliburton and Drummond, 1917.
Hardened fats, animal or vegetable origin	0			" " " "
CEREALS, PULSES AND BREAD				
Barley	+	++	0	Steenbock, Kent and Gross, 1918; Cooper, 1913; Fürst, 1912.
Germinated pulses or cereals	+	++	++	Fürst, 1912; McClendon, Cole and Engstrand, 1919; Weill, Mouriquand and Peronnet, 1918.
Lentils, dried		+++		Cooper, 1913.

Classes of Foodstuff	Fat Soluble Vitamin A	Antineuritic Vitamin B	Antiscorbutic Vitamin C	References
Maize (corn)	O in white + in yellow	+++		Steenboek and Boutwell, 1920; McCollum, Simmonds and Pitz, 1916; Hogan, 1916; Voegtlin, Lake and Myers, 1918; Steenboek, 1919.
Millet seed	++	++		McCollum, Simmonds and Pitz, 1917.
Navy beans		+++		
Oats (oatmeal)	+	++		McCollum, Simmonds and Pitz, 1917; Holst and Fröhlich, 1907.
Peas, dried		++	O	McCollum, Simmonds and Parsons, 1919.
Peas, fresh	+	+	+++	Drummond, 1918.
Rice, polished	O	O	—	Eijkman, 1897; McCollum and Davis, 1915.
Rice, whole grain	+	++	—	Chick and Hume, 1917.
Rye	+	++		McCollum, Simmonds and Pitz, 1915.
Soy beans	++	++		Osborne and Mendel, 1917.
Wheat embryo	++	+++	+	McCollum, Simmonds and Pitz, 1916; Voegtlin and Myers, 1918; Hess, 1916.
Wheat bran	—	+	—	Chick and Hume, 1917; Voegtlin, Lake and Myers, 1918; Osborne and Mendel, 1919.
Wheat kernel	+	++	O	McCollum, Simmonds and Pitz, 1915; Voegtlin and Myers, 1918; Osborne and Mendel, 1919.
Wheat bread, white	O	O	O	Voegtlin and Myers, 1918; Holst and Fröhlich, 1912.
Wheat bread, whole meal	+	++	O	Voegtlin and Myers, 1918; Voegtlin, Lake and Myers, 1918.
White wheaten flour, pure	O	O	O	Voegtlin, Lake and Myers, 1918; Voegtlin and Myers, 1918.
EGGS				
Egg yolk	+++	++	O	McCollum and Davis, 1914; Cooper, 1913; Hess and Unger, 1918.
MEAT, FISH, ETC.				
Brain	+	++		Cooper, 1913; Osborne and Mendel, 1918.
Codfish	—	+		Drummond, 1918.
Heart muscle (pig, ox)	++	++		Cooper, 1913; Osborne and Mendel, 1918.
Kidneys	++	+		McCollum and Davis, 1915.

TABLE OF VITAMINS—*Continued*

Classes of Foodstuff	Fat Soluble Vitamin A	Antineuritic Vitamin B	Antiscorbutic Vitamin C	References
Lean meat (beef, mutton, etc.)	+	+	+	Osborne and Mendel, 1917; Cooper, 1913.
Liver	++	++	+	" " " " " "
Sweetbreads	+	++		Eddy, 1916.
MILK, CHEESE, ETC.				
Cheese	O	O		Cooper, 1914.
Cheese, whole milk	+			
Milk, cow's whole, raw, fresh	++	+	+	Osborne and Mendel, 1918 and 1920; Chick and Hume, 1919; Hart, Steenbock and Smith, 1919.
" skim powder	O	+		McCollum and Davis, 1915.
" dried whole	less than ++	+	less than +	Barnes and Hume, 1919.
" pasteurized, 24 hrs. old			trace	Hess, 1916.
" boiled, whole	undetermined	+	less than +	
" condensed, sweetened	+	+	O	Hart, Steenbock and Smith, 1919.
" human fresh		less than +		Gibson, 1913.
VEGETABLES AND FRUITS				
Bananas	+	+	trace	Sugiura and Benedict, 1919; Lewis, 1919.
Beans, dried		++		Grijns, 1901.
Beets		++	less than +	Osborne and Mendel, 1919.
Cabbage, fresh raw	++	+	+++	Osborne and Mendel, 1920; McCollum, Simmonds and Pitz, 1916; Holst and Fröhlich, 1912.
" " cooked		+	+	
" dried	++	+	trace	Osborne and Mendel, 1919.
" canned			trace	
Carrots, raw and cooked	+	++	+	Osborne and Mendel, 1920; Denton and Kohman, 1918; Steenbock and Gross, 1919; Hess and Unger, 1919.
Lemon juice, fresh			+++	Chick, Hume, Skelton and Smith, 1918.
" " preserved			++	Chick, Hume, Skelton and Smith, 1918.
Lime juice, fresh			++	Chick, Hume, Skelton and Smith, 1918.
" " preserved			trace	Chick, Hume, Skelton and Smith, 1918.
Lettuce	++	+		Steenbock and Gross, 1920.
Nuts	trace	++		Carjori, 1920; Daniels and Loughlin, 1918.
Onions		++	+++	Osborne and Mendel, 1919.

Classes of Foodstuff	Fat Soluble Vitamin A	Antineuritic Vitamin B	Antiscorbutic Vitamin C	References
Orange juice, fresh			+++	Givens and McCluggage, 1919; Hess and Unger, 1919.
Parsnip		++		Steenbock and Gross, 1919.
Peanuts	+	++		Daniels and Loughlin, 1918.
Potatoes, raw	+	+		Osborne and Mendel, 1920; Steenbock and Gross, 1919.
" cooked	+	+	+	McCollum, Simmonds and Pitz, 1918.
Rutabaga	trace	++		Steenbock and Gross, 1919.
Spinach	++	++	+++	Osborne and Mendel, 1919; Steenbock and Gross, 1920.
" dried	+++	++		Osborne and Mendel, 1919.
Tomato	++	++	++	Osborne and Mendel, 1919 and 1920; Hess, 1918; Givens and McCluggage, 1919.
" canned			++	Hess and Unger, 1918.
Turnips		++		Osborne and Mendel, 1919.
MISCELLANEOUS				
Alfalfa	++	++		Osborne and Mendel, 1919; McCollum, Simmonds and Pitz, 1916; Steenbock and Gross, 1920.
Clover, dried	+++	++		Osborne and Mendel, 1919.
Cod testes	+			McCollum and Davis, 1915.
Timothy, hay	+++	++		Osborne and Mendel, 1919.
Yeast, dried		+++	—	Cooper, 1913.
" autolyzed		+++	0	Seidell, 1916; Hess, 1916.

Recent work indicates that the growth of unicellular organisms, such as yeast and certain bacteria, is dependent upon a supply of vitamin. As a result of Bachmann's observations (1919), Williams (*a*) (*b*) (1919, 1920) has elaborated a promising method for the quantitative estimation of the antineuritic vitamin, based upon the observation that the growth of yeast is proportional to the vitamin content of the medium. The reliability of this method should, however, be fairly established before its general adoption for work of this kind.

Drummond (*b*) (1917) has made observations on the influence of a deficiency of fat-soluble or antineuritic vitamin in the diet on the growth of tumors. He comes to the conclusion that a lack of the fat-soluble vitamin has no effect, whereas the absence of the antineuritic vitamin causes a certain amount of inhibition.

As a concluding remark it may be said that the work of this last decade has resulted in numerous discoveries regarding the physiological and pathological significance of vitamins. Although some facts have been pretty firmly established, this does not hold for all observations made in this field. As a matter of fact, the study of vitamins is still in its infancy and sweeping generalizations, as so often made in scientific literature, do not serve a good purpose. We are fairly well informed as to the distribution of the three vitamins in the more important foodstuffs. Further progress will largely depend on the chemical isolation of these substances, a phase which so far has attracted the attention of a relatively small number of investigators.

SECTION II

A Normal Diet . . . . . *Isidor Greenwald*

Introduction—The Diet of Primitive Peoples—Food and Civilization—Crop Failures and Famine—Criteria of Adequacy of Diet—Relative Impotence of Certain Foods—Dietary Studies—Manner of Conducting Studies and of Calculating Results—Studies of Entire Countries and Cities—Studies upon Individuals and Groups on Fully Chosen Diets—Influence of Climate and Season upon Food Consumption—Influence of Work—Amount of Protein—Amount of Fat—Ash Constituents—Changes in Food Habits within Recent Times—Vegetarian—Protein Minimum and Optimum—Neumann's Experiments—Chittenden's Experiments—Fisher—McCay—Fat Minimum—Carbohydrate Minimum—Minimum of Ash Constituents—Undernutrition—Conclusion.

A Normal Diet

ISIDOR GREENWALD

NEW YORK

Introduction

The Diet of Primitive Peoples.—From as early a time as we can discern anything of the life of man we find that this has been an almost unceasing struggle for food, for enough to enable him to satisfy his wants. So far as we can judge from the remains, from the habits of the animals most closely resembling man, and from those of backward or undeveloped peoples, the diet of primitive man consisted of whatever that was edible that he could secure. The Mincopies, or inhabitants of the Andaman Islands, regarded as among the most primitive, or lowest in scale of civilization, of the human race, live chiefly on mangoes and other fruit, shellfish and an occasional small wild pig. The Fuegians, another primitive people, subsist almost entirely on shellfish. Heaps of shells, supposed to be the remnants of the middens of primitive man, are found in different parts of the world (Avebury, Tyler). Scott-Elliott believes the food of Pleistocene man to have consisted of nuts, fleshy fruits, small birds' eggs, honey, insects and shellfish. There is no evidence that man, except under the influence of a religious or pseudo-scientific inhibition, dating from very recent times, ever voluntarily restricted himself to a purely vegetarian diet. On the contrary, amongst such peoples as the Fuegians, and in the nomadic and pastoral stages of civilization, his diet was almost exclusively of animal origin. The relative importance of vegetable and animal foods varied with their relative availability. Both kinds were frequently eaten raw but the earliest evidences and the descriptions of the life of the most primitive of peoples indicate that, from a very early stage, man has cooked some of his food, at least occasionally and as opportunity offered. Man has, indeed, been called "the cooking animal."

Food and Civilization.—The development of civilization depended very largely upon the kind of food man was able to secure. Semple states: "In Australia, the lack of a single indigenous mammal fit for domestication and of all cereals blocked from the start the pastoral and agricultural development of the native." The American continents were more fortunate for, with beans, maize and pumpkins, it was possible for a

limited agriculture to develop. It is, perhaps, in North America that one can see most clearly how the nature of the available supply affected the food habits of the natives. The Indians of the plains were essentially hunters and lived largely on the results of the chase. In the east, agriculture was fairly well established, among some tribes at least, and maize, beans, pumpkins and other plants constituted a very considerable part of the diet. But it was in what is now the southwestern part of the United States and in Mexico that the greatest progress in agriculture occurred and it was there that the highest civilization developed. In contrast with the tribes of these sections, all of whom were fairly well fed, we find the stunted and emaciated Indians of the northern Rocky Mountains, denied both the chase of the buffalo and the cultivation of maize.

It was in the Old World that animals susceptible of domestication, especially those suited for a nomadic life, were most numerous and it was there that pastoral civilization reached its fullest development. Cereals and legumes were also abundant and furnished the basis for a more settled life. It was no longer necessary for so much time to be given to the obtaining of food; more could be devoted to other wants, the satisfaction of which is the characteristic of civilization.

Crop Failures and Famine.—All through the ages, such margin as separated man from an actual food shortage has been very narrow. Famine has always been a very present menace, as the liturgies of the churches abundantly testify. The yield of the staple foods, from year to year, is very uncertain even at this time. With a population dependent upon closely neighboring sources of supply, any failure of the accustomed yield means scarcity and even starvation. It was only with the development of transportation, particularly in the latter part of the nineteenth century, that a fairly regular food supply could be assured to most of mankind. Even then, famine was not unknown in Russia, China and India. With the breakdown of commerce and transportation and the withdrawal of large areas of land and of millions of men from food production as a result of the world war, famine has reappeared in regions from which we had once believed it banished.

Even in so large and fertile country as our own and one so well provided with railroads and other means of communication, the failure of a staple crop may involve, if not deprivation of sufficient food energy, a failure to secure sufficient of the less well-recognized dietary constituents. To quote from Hess(*e*) (1920): "It is important for us to realize that we are still dependent on the annual crops for our protection from scurvy; in other words, the world is leading a hand to mouth existence in regard to its quota of antiscorbutic food. The truth of this condition has been realized for Ireland, sadly illustrated by numerous epidemics, notably the great epidemic of 1847 reported by Curran. It was demonstrated by the outbreaks of scurvy in Norway in 1904 and 1912 and was brought to the atten-

tion of many in the United States in the spring of 1916. In this year our potato crop fell far below the normal, with the result that scurvy appeared in various parts of the United States, especially in institutions."

Short of actual famine and the acute distress and suffering due to occasional crop failures, the development of man may be hampered by chronically insufficient or improper food. The case of the RockyMountain Indians has already been mentioned. Ripley regards the low stature and poor physical condition of the natives of the Auvergne plateau in southern France as due to the impossibility of obtaining an adequate diet from the soil of that region. Removed from the district while young, the children develop normally.[1] The peasants of the Abruzzi seem to furnish another illustration of the damaging effect of an unsatisfactory diet upon a whole people. These peasants are amongst the shortest in Italy but when the young men enter the army and receive a more adequate diet they grow rapidly and this growth is greater than for any others except the men from a few districts in which a similarly unsatisfactory diet is employed. (Albertoni and Rossi(*b*), 1908; Lichtenfelt, 1912, page 34.) The damaging effects of malnutrition in cities have been much discussed. While these are generally considered to be occasional, rather than general, there is some evidence that they may affect a very considerable proportion of the population and may, indeed, alter the physical characteristics thereof. Thus, Collis and Greenwood regard it as likely that the short stature of the cotton operatives in Lancaster is due to a deficient diet. The nature of some of these supposedly unsatisfactory diets and the criteria of their inadequacy will be discussed later.

Definition of "Normal."—It is obvious that in any given country and at any given period, the people living there and then must regard their diet as the normal. It is the "usual, common or ordinary" as the dictionary defines "normal." But, to the physician, physiologist or hygienist the word "normal" relates to good health and the "usual, common or ordinary" is employed only as a means of ascertaining what is to be considered healthy. A normal diet must be capable of maintaining man in good health and our conception of a normal diet will become more definite with increasing knowledge of what is to be considered good health and of the relation between diet and health. It may, then, fairly be questioned if the "usual, common or ordinary" diet, as it obtains to-day, even amongst those most free to chose is really a "normal" diet.

In this chapter an attempt will be made to discuss the subject from both points of view. The nature and amount of the food materials made use of by civilized man in different parts of the world will first be considered. Then the results of more detailed studies upon the diet of groups and of individuals in different climates, engaged in different occupations and of different economic status will be presented. An attempt will be

[1] Ripley gives Collignon as his authority. I have not been able to find the original.

made to point out certain properties common to all or most of such diets, to discuss the significance of the differences and to indicate wherein the evidence shows some of the diets to be inadequate. Finally, the question of a possible improvement in our dietary habits will be discussed and the various measures proposed for this purpose will be considered.

Criteria of Adequacy of Diet.—It is obvious from the preceding chapters that the adequacy of a diet may be judged from many different aspects; energy yield, nature and amount of protein, nature and content of inorganic material, etc. Probably, the most essential of these is energy yield. Unless the diet be restricted to a certain few materials, it is, if sufficient in energy yield, sure to contain a considerable, even if not entirely adequate, amount of protein, inorganic matter, etc. However, it should be clearly recognized that this primacy of energy requirement may be due largely to the fact that our means for determining the energy

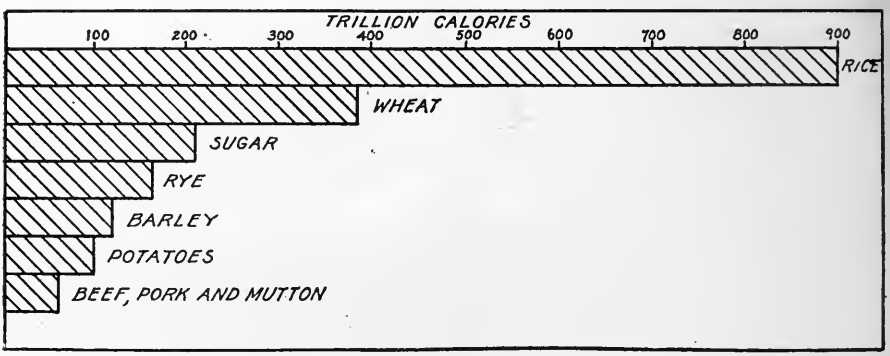

Chart I.—Total food value of the chief world foods expressed in calories. Rice, wheat and sugar are practically all consumed as human food. Some of the rye and barley is distilled or used for animal food. A considerable part of the potato crop is used for industrial purposes. Data from G. K. Holmes, *The Meat Situation in the United States*, Dept. of Agriculture, Office of the Secretary, Report No. 109. Figure from G. B. Roorbach, *The World's Food Supply*, Proceedings of the American Philosophical Society, Philadelphia, 1918, Vol. 57, pp. 1-33.

content of the food and the energy requirements of the body are the better developed. It may yet be found that man's desire for food is directed primarily to securing, not a sufficient supply of energy, nor even of protein, but perhaps of some inorganic constituent or of some as yet unknown or imperfectly recognized organic substance of the kind variously known as vitamines, protective substances, food hormones, etc. Thus Osborne suggested that the beneficial results of exercise may be due, in part, to the ample supply of these substances secured as a consequence of the hearty appetite thus produced. But, for the present, we will consider food primarily as a supplier of energy, then of protein and only secondarily of other constituents.

Relative Importance of Certain Foods.—The amount of energy contributed annually to the world's food by the more important food materials

has been calculated by Holmes to be, in trillion calories: rice, 900; wheat, 382; sugar, 209; rye, 164; barley, 119; potatoes, 98.6 and meat 62.4. The chart on page 362 was prepared by Roorbach from Holmes' figures. Unfortunately, Holmes does not cite his authorities, and the figure for sugar appears remarkably high. The relative importance of the different foods shown by these figures is, however, true for no one country. In some parts of the East, rice is even more largely the predominant food and, on the other hand, the consumption of meat is concentrated in a very few countries.

The figures in Table I are taken from Holmes and show, in pounds, the annual per capita consumption of meat and meat products. No data are reported for China, India and Japan but the consumption of meat there is known to be small. The amount of meat used, per person, is greatest in the meat-raising countries, in all of which the density of population is rather low. (Chart II is taken from Roorbach.)

TABLE I.—CONSUMPTION OF MEAT AND MEAT PRODUCTS (BEEF, MUTTON AND PORK) PER CAPITA OF POPULATION.—*Data from Holmes.*

Country	Year	Pounds	Country	Year	Pounds
Argentine	1899	140	Netherlands	1902	70
Austria-Hungary	1890	64	New Zealand	1902	212.5
Australia	1902	262.6	Norway	1902	62
Belgium	1902	70	Poland (Russian)	1899	62
Canada	1900	109	Portugal	1899	44
"	1910	137	Russia (except Poland)	1899	50
Denmark	1902	76	Spain	1890	49
France	1892	77	Sweden	1902	62
"	1904	79	Switzerland	1899	75
Germany	1894	88	United Kingdom	1893	112
"	1904	112.7	" "	1906	119
"	1913	111.8	United States	1900	182
Greece	1899	68	" "	1909	171
Italy	1901	46.5			

As the population increases, pasture land is put under cultivation, the production and consumption of meat fall and the use of the cereals and other foods increases. A fairly high consumption of meat may be maintained, and even increased, as in Germany and Great Britain, in spite of an increasing population in a manufacturing and trading community if the level of wealth is sufficiently high to secure the importation of meat or of concentrated feeding stuffs for animals. But, as a rule, the importance of meat in the diet diminishes as the population increases and such meat as is consumed falls chiefly to wealthy and powerful classes.

The medieval laws restricting the taking of game seem to have had their origin not so much in the desire to secure sport to the nobility as to secure to them an ample supply of meat, or of certain kinds of meat. (Lichtenfelt(*c*), 1913.) The same predominating use of meat by the wealthier and more powerful classes obtains to-day in all countries except those in

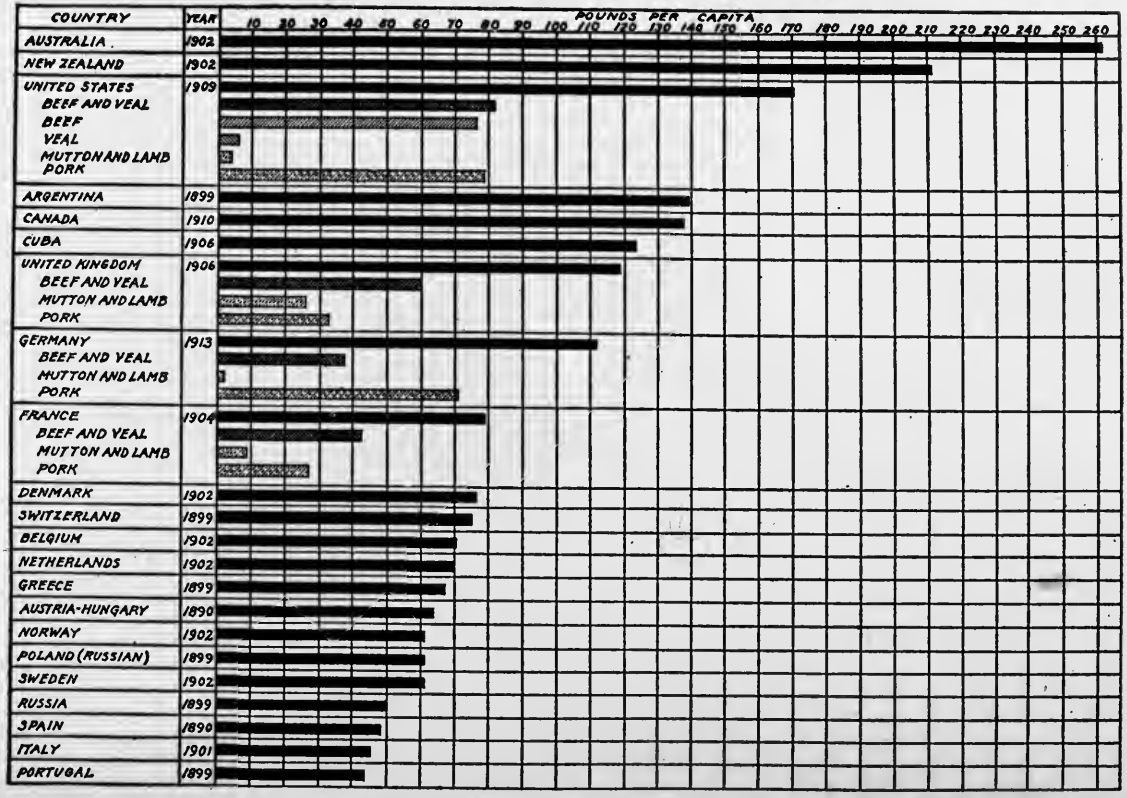

Chart II.—Per Capita Consumption of Meat. Data from G. K. Holmes, *The Meat Situation in the United States*, Dept. of Agriculture, Office of the Secretary, Report No. 109. Figure from G. B. Roorbach, *The World's Food Supply*, Proceedings of the American Philosophical Society, Philadelphia, 1918, Vol. 57, pp. 1-33.

which meat-raising is one of the chief industries. In 1903, the per capita consumption of meat in Great Britain was, among artisans, laborers and mechanics, two pounds per week; among the lower middle classes, paying from $75 to $125 annual rental, 2.5 pounds; in the middle classes, 3.5 pounds, and amongst the upper classes 5.75 pounds. (Lusk(*i*), 1918.)

As can be seen from Holmes' figures, the cereals furnish most of man's food. Certain few are of particular importance. In the earliest periods, barley was the predominating or only cereal. In Europe, barley was supplanted by oats and by rye and these, in turn, were in great part displaced by wheat. In eastern and southern Asia the supplanting cereal was rice.

In order to make them more available as food, man early learned to break and grind the grains, to soak the fragments in water and to cook this porridge. Cereals prepared in this way are to this day a very important and even a major part of the food of the people in many lands. Familiar examples are the boiled rice of the East, the oatmeal porridge of Scotland, the maize polenta of Italy and, in a slightly modified form, the many flour soups and cooked dough dishes of central Europe. It probably did not take man long to discover that the uncooked mixture of cereal and water could be dried in the sun or over the fire and that this then furnished, with or without cooking, a readily available, yet durable source of food. Present day examples are spaghetti, etc., noodles of all kinds, the oat and barley cakes of northern Europe and the unleavened bread of much of Asia and of other parts of the world. The preparation of an actual bread came much later and is, in fact, a matter of comparatively recent and local development. For this purpose neither rice nor maize can be used alone and rye and wheat are immensely superior to barley. This superiority depends upon the peculiar properties of the proteins of wheat and rye flour. These form a sticky, extremely tenacious mass when mixed with water. This mass holds the starch, etc., firmly, imprisons the carbon dioxid formed by fermentation and thus produces a light, firm loaf. This will hold its shape in spite of considerable handling and can be preserved with comparatively little change for a considerable time and even indefinitely. It is this superiority of wheat and rye for bread making that has caused them to so largely supplant the other cereals as sources of human food. Wheat bread is generally preferred to rye because of its color and texture and, by some, because they find the taste more agreeable. But there are many, chiefly those accustomed to it from early life, who prefer the taste of rye bread. At any rate, it is still *the* bread of most of eastern and central Europe, except in the larger cities. (See Table III.)

However, there seems to have been, until the outbreak of the war, a gradual displacement of rye by wheat. To a considerable extent, no doubt, this was due to the increasing proportion of the population living

in cities, which always lead in the consumption of wheat as compared with rye, barley or oats. But Sherman(*b*) (1918) has collected figures showing that in Russia in the period from 1894 to 1899, there were 1.82 bushels of wheat and 4.76 bushels of rye consumed per person per annum. During the following five years, these figures were 2.46 and 4.78, respectively, and from 1911 to 1913 were 2.86 and 4.47. The magnitude of these changes in a country with, relatively, so small an urban population indicates that the use of wheat was increasing in the country as well as in the cities.

Dietary Studies

Manner of Conducting Studies and of Calculating Results.—The amount and composition of the food consumed per person may be determined in various ways. As in the calculations of Sherman and of Holmes, the total amount of food raised in and imported into a given area, less that exported and used otherwise than as human food, may be divided by the number of people. The method is, at best, only an approximation but it serves very well to indicate the relative importance of the different food materials. Next, studies may be made of groups such as families, eating clubs, public institutions, military and naval organizations, etc., in which the total amount of food is weighed and, with or without deduction for waste, is divided by the total number of people participating. Finally, the food consumed by an individual may be weighed.

The composition of the food may be calculated in different ways. For such gross calculations as those relating to the food consumption of an entire city or country, it is obvious that only the average of a considerable number of analyses can be used. In the other cases, the same procedure may be followed but it is also possible, and preferable, to secure sufficient of most of the materials to last through all, or a considerable part, of the experiment and to analyze representative samples of these. Still greater accuracy may be obtained by taking to the laboratory and analyzing a composite sample of the food consumed, weighed as served, and mixed in exactly the same proportion as consumed.

Assuming the trustworthiness of the subjects, many factors influence the accuracy and significance of the results. Studies made under laboratory conditions with accurate weighing and analysis of the food are the most accurate but are obviously expensive and difficult to make in large number for a long period. Studies made in the home can be carried out in larger number, can be continued for a longer period and come nearer to "normal" conditions but the accuracy of the weighings and the applicability of the analytical data employed are more questionable. Daily and seasonal variations in food consumption must also be considered. The

former are generally neutralized in periods of a week or longer but the latter may be appreciable, particularly in agricultural communities and in others in which transportation and storage facilities have not been well developed.

The results of observations upon adults of either sex may be reported directly as so much per person, per kilo or per square meter of body surface. With groups including both sexes or adults and children, it is essential to have some unit in which to express the results. Omitting the periods of pregnancy and lactation, women have a lower food requirement than men because of smaller body weight, lower basal metabolism per kilo, and, as a rule, less mechanical work performed. Children may eat less than adults but consume more per kilo of body weight.

Choice of Factor for Calculating Food Consumed "Per Man."— From time to time, various methods have been proposed for converting observations made on groups including women or children to a "per man" basis. The table (Table II) on page 368 is a compilation of the more important of these, the food requirement of a man of average weight (70 kilos or 154 pounds) engaged in a moderate amount of work being taken as 100. The first six columns are copied from the report of the Eltzbacher commission. This was organized in 1914 to survey the food resources and requirements of the German nation. It included in its membership both Zuntz and Rubner. For the value of the food energy requirement of the German people, they used the average of the results calculated by each of the six series of factors. Most other investigators and reporters have used Atwater's factors and generally the earlier set. These are certainly in error in giving too low a value to the food requirements of growing children. In fact, recent investigations (Gephart, Holt and Fales) indicate that all the sets of factors used by the Eltzbacher commission and by others are erroneous and that rapidly growing boys and girls require more food than adults. Holt and Fales have tabulated the energy requirements of children at different ages. They regard that of an adult male as 3265 calories per day. From their figures, the author has calculated the factors found in the last column, which are, for American children, probably more accurate than any others hitherto used. There must, of course, be variations in the value of the factors in different parts of the world and among different races due to the variation in the age of attaining maturity and the rapidity of growth at any given age.

The factor for women is generally taken as 80 (man = 100) though in compiling the report of the U. S. Commissioner of Labor in 1903 it was set at 90 and Rubner (Eltzbacher commission) considered it to be 100. Two series of Russian observations, cited in Table IV, yield the ratios 81.5 and 88, respectively. Slosse and Waxweiler in a series of 6 comparisons obtained values for 73 to 95, average 85. On the other hand, Sundström (1908), in his series of observations on Finnish men and women, found it

TABLE II.—SCALES FOR CONVERTING FOOD REQUIREMENT OF WOMEN AND CHILDREN INTO "MAN EQUIVALENTS."

Authority	A Engel [1]		B Rubner [2]	C Atwater [3]		D Zuntz [4]		E American Households [5]		F Danish Statistics [6]		G Atwater [7]		H Sundstrom [8]		I Committee of Royal Society [9]		J Pearl [10]		K Holt & Fales [11]	
Age	m. and f.		m. and f.	m. and f.		m. and f.		m. and f.		male	female	m. and f.		m. and f.		m. and f.		m. and f.		male	female
Under 1	28.6			30		20		15		22	17.6	30		10							
1	31.4		21.8	30		30		15		22	17.6	30		15		40		50		29	29
2	34.3		29.7	40		40		15		30	24.0	40		20		40		50		35	34
3	37.1		35.4	40		40		15		36	28.8	40		25		40		50		39	38
4	40.0		38.8	40		40		40		39	31.2	40		30		40		50		42	40
5	42.9		42.3	40		50		40		43	34.4	40		35		40		50		46	43
6	45.7		43.7	50		50		40		44	35.2	50		40		50		77		49	47
7	48.6		45.0	50		50		75		45	36.0	50		45		50		77		53	51
8	51.4		46.4	50		50		75		47	37.6	50		50		50		77		59	56
9	54.3		47.8	50		50		75		48	38.4	50		55		50		77		65	61
10	57.1		49.2	60		75		75		50	40.0	60		60		60		77		71	67
11	60.0		54.4	60		75		90		55	44.0	60		65		60		77		77	77
12	62.9		59.6	60		75		90		60	48.0	60		70		60		77		84	88
												male	female	male	female						
13	65.7		65.0	60		75		90		66	52.8	80	70	75	70	60		77		93	98
				male	female											male	female	male	female		
14	68.6		70.6	80	70	75		90		71	56.8	80	70	80	70	80	70	100	83	104	102
						male	female	male	female												
15	71.4		73.1	80	70	100	80	100	90	74	59.2	90	80	85	70	80	70	100	83	118	99
16	74.3		75.7	80	70	100	80	100	90	76	60.8	90	80	90	70	100	80	100	83	125	97
17	77.1		78.4	80	70	100	80	100	90	79	63.2	100	80	95	70	100	80	100	83	121	94
18	80.0		81.6	100	80	100	80	100	90	82	65.6	100	80	100	70	100	80	100	83	114	90
19	82.9		100	100	80	100	80	100	90	100	80	100	80	100	70	100	80	100	83	100	81
20	85.7		100	100	80	100	80	100	90	100	80	100	80	100	70	100	80	100	83	100	81
	male	female																			
21	88.6	85.7	100	100	80	100	80	100	90	100	80	100	80	100	70	100	80	100	83	100	81
22	91.4	85.7	100	100	80	100	80	100	90	100	80	100	80	100	70	100	80	100	83	100	81
23	94.3	85.7	100	100	80	100	80	100	90	100	80	100	80	100	70	100	80	100	83	100	81
24	97.1	85.7	100	100	80	100	80	100	90	100	80	100	80	100	70	100	80	100	83	100	81
25–59	100	85.7	100	100	80	100	80	100	90	100	80	100	80	100	70	100	80	100	83	100	81
60 and over	100	85.7	100	100	80	90	80	100	90	100	80	100	80	100	70	100	80	100	83	100	81

[1] Engel, 1895. [2] Not known. [3] Atwater, 1895. Used in all investigations of Atwater and coworkers. [4] Not known. [5] U. S. Commissioner of Labor, Report, 1903. [6] Heiberg and Jensen, 1911. [7] Atwater, 1903. [8] Sundstrom, 1908. [9] Committee of Royal Society, 1917. [10] Pearl, 1920. [11] Holt and Fales, 1921.

The report of the Eltzbacher commission, from which the first six columns are taken, does not give references, but the above are probably correct.

to be only 70. His factors for the food consumption of growing children were also rather low. Probably these low values are due to the fact that Sundstrom's adult male subjects were all engaged in hard muscular work, rather more severe than the standard "moderate work" used by others whereas the women and children were not so unusually active. If this reasoning is correct, Sundstrom's values should be increased by from 10 to 20 per cent.

Results Reported as Food Consumed Not that Supposed to be Absorbed. —Some of the observers whose results are summarized in Table III and IV have reported their findings in terms of "available" calories and "digestible" protein, the values being calculated with the aid of factors obtained in metabolism experiments in which the nitrogen content and energy value of the feces have been regarded as being due to undigested or unabsorbed food. This does not, to the present writer, appear to be justified. The percentage of nitrogen in the feces is approximately the same no matter what the diet but the amount of feces formed and, consequently, the amount of nitrogen excreted therein is greater with vegetable material than with animal. However, the relation of fecal nitrogen to food nitrogen after the ingestion of specific foods is not a constant but depends a great deal upon the individual, upon the method of preparation of the food and the nature of the other constituents of the diet. Thus, Albertoni and Rossi(*a*) (1908) found that the addition of meat to the customary vegetarian diet of Italian peasants, although increasing the total nitrogen of the food, diminished not only the relative but also the absolute amount of nitrogen in the feces. On their customary diet containing 75.7 grams protein, three men excreted a daily average of 3.21 grams nitrogen in the feces. On diets containing 98.7 grams protein, of which only 21.2 grams was meat protein and the remainder was derived from the customary food, the nitrogen in the feces was 2.94 grams; and with 111.13 grams protein, of which only 40.8 grams were derived from meat, the fecal nitrogen was 2.16 grams. Similar results were obtained with two women, the figures being 55.8 grams protein intake without meat with 2.71 grams nitrogen in the feces and 92.6 grams protein, of which 43.3 grams were meat, on the experimental diet, with only 1.533 grams nitrogen in the feces. A similar though much less marked effect of added glucose was observed by Neumann (*d*) (1919) who found that on a diet of 1000 grams of whole rye bread, his feces contained 2.52 grams nitrogen daily. Upon adding 300 grams glucose to the diet, the fecal nitrogen fell to 2.44 grams and, after increasing the glucose intake to 500 grams, to 2.41 grams.

Again, Hindhede(*d*) (1914) found that the addition of plums to a bread diet increased the nitrogen of the feces by an amount greater than the total nitrogen of the plums. Hindhede regarded this as evidence of interference with protein absorption but, since there was no such evidence of interference with carbohydrate or fat absorption, it seems possible that the

TABLE III.—AMOUNT AND NATURE OF

Date	Country	Authority	Per "Man" per Day				Scale used to convert population into "man equivalents"[2]	Percentage Distri			
			Protein grams	Fat grams	Carbohydrates grams	Calories		Meat[8]	Milk and products	Wheat	Other grains
1912–7	United States..	Pearl.....	121	169	542	4290	J	25.5	20.4	28.9	7.2[26]
			114[3]	127[3]	433[3]	3424[3]					
1909–13	Great Britain and Ireland	Committee of Royal Society	113	130	571	4009	I	34.8	13.7	34.6	3.6
1894	Germany.....	Lichtenfelt	123	94	528	3800[4]	A	22.9	13.7	40.5	
		(1898)	(104)	(81)	(504)	(3336)[1]					
1907	Germany (rural)	Claassen (a)..	(146)	(195)	(679)	(5193)[1]	66.7%	(16.8)	(25.0)	(2.5)	(31.8[10])
	(urban)	"	(98)	(141)	(467)	(3633)[1]	66.7%	(35.4)	(20.6)	(13.8)	(21.7[12])
1912–3	Germany.....	Eltzbacher Commission per capita...	(93)	(106)	(531)	(3642[5])[1]	average of A-F=76.2%	(23.5)	(21.2)	(16.6)	(18.1)
		per man	(122)	(139)	(699)	(4777[5])[1]					
1890–9	Paris.....	Gautier, per capita.....	107[6]	57	314	2606[7]		43.6	14.1	28.0[14]	
		per man	140	73	408	3385[7]	77%				
1886	Italy.....	Lichtenfelt (b)	151	78	550	3586	A				
		(1903)	(138)	(67)	(524)	(3448)[1]					
1904	Russia.....	Sherman(1918) per capita...	90			2997		11.1	3.4	70.00	
		per man	117			3880	77%				

[1] Figures in parentheses represent "digestible" nutrients. [2] See Table II. [3] After deducting waste of 5% protein, 25% fat and 20% carbohydrate. [4] Includes 254 calories from alcohol. [5] Includes 173 calories per capita, or 228 "per man," from alcoholic beverages, or 112, and 147, respectively, from alcohol. [6] Gautier gives total as 102 but total of individual entries is 107.5 grams. [7] Includes 354 and 460 calories, respectively, from alcohol. [8] Includes fish, poultry and eggs. [9] 10.5% from

plums stimulated the excretion of nitrogen into the intestine. Mosenthal (*a*) (1911) found that in dogs on a mixed diet, which would be a high protein diet for man, the excretion of nitrogen into the intestine was about 35 per cent of the intake and that 25 per cent was later reabsorbed. Hindhede's results could be explained by an increased excretion of such nitrogen without compensatory reabsorption. It is quite possible and even probable that such nitrogen has not been completely metabolized and therefore represents as real a loss to the body as if it were unabsorbed food nitrogen but the fact has not yet been fully established. It is just possible that the material excreted into the intestine is as truly a waste product as urea or any other constituent of the urine. However that may be, it is evident from the observations of Albertoni and Rossi and of Neumann that "factors of digestibility" derived from certain experiments cannot properly be used in calculating "digestible protein" under different conditions. See also Rubner(*aa*) (1918). Therefore, the discussion in this chapter, unless the fecal or urinary nitrogen has actually been determined in the particular observation under discussion, will, unless specifically otherwise noted, be based upon the nitrogen and energy content of the food, the latter being calculated by the use of Rubner's factors, 4.1 calories per gram of protein or carbohydrate and 9.3 per gram of fat.

FOOD CONSUMED IN DIFFERENT COUNTRIES[1]

BUTION OF PROTEIN				PERCENTAGE DISTRIBUTION OF CALORIES								
Potatoes	Other vegetables	Nuts and fruits	Other foods	Meat[8]	Milk and products	Other fats	Wheat	Other grains	Potatoes	Other vegetables	Nuts and fruits	Sugars	Other foods
3.1	2 7	2.0	0.3	24.1	15.3	4.0	25.9	8.8[18]	3.4	2.0	3.1	13.2	0.3
8.1	3.7	0.7	0.6	19.6	12.7	1.8	30.9	3.9	12.5	1.9	2.3	12.6	0.1
6.3	13.7[9]	0.3	2.6	16.2	11.0		43.5		9.3	6.3	0.8	3.9	9.1[19]
(9.4)	(13.3[11])	(1.2)		(24.9)	(15.1)		(2.9)	(31.4[20])	(15.3)	(5.9)	(2.8)	(1.8)	
(4.8)	(3.5)	(0.3)		(25.4)	(17.2)		(15.8)	(22.4[21])	(7.4)	(1.4)	(0.7)	(9.6)	
(8.0)	(10.4[13])	(1.0)	(1.2)	(17.3)	(13.1)	(1.9)	(16.6)	(22.2[22])	(11.7)	(4.3)	(2.4)	(5.4)	(5.1[23])
1.2[15]	13.0[16]	0.1		15.2	15.1	1.1	37.1[14]		3.4[15]	7.9	0.8	6.0	13.6[24]
9.0	6.3[17]	0.1		4.7	2.2	1.9	75.3		10.2	3.1[25]	0.6	2.2	

legumes. [10] 31.7% from rye. [11] 5.1% from legumes. [12] 21.0% from rye. [13] 5.4% from legumes. [14] Does not include rice. [15] Includes rice. [16] 8.8% from legumes. [17] 5.3% from legumes. [18] 7.0% from maize. [19] 6.7% from alcohol. [20] 31.2% from rye. [21] 21.1% from rye. [22] 15.2% from rye. [23] 4.8% as alcoholic beverages, 3.1% as alcohol. [24] 13.6% from alcohol. [25] 2.4% from legumes. [26] 5.55% from maize.

Studies of Entire Countries and Cities

The great part played by food, or by the lack of it, in the World War, was responsible for very careful studies of the food statistics of some of the countries involved. Perhaps the most complete of these that has been published is that made by Pearl for the United States. In Table III, there are presented figures taken or calculated from Pearl, from a report of a committee of the Royal Society of London and from the report of the Eltzbacher commission. There are also included figures obtained from the reports of Lichtenfelt(*a*)(*b*) (1898, 1903) on food consumption in Germany in 1894 and in Italy in 1886, of Claassen(*a*) for the urban and rural population of Germany in 1909, of Sherman(*b*) (1918), for Russia in 1913 and of Gautier for Paris from 1890 to 1899. These last, obtained from the records of the octroi, or customs collected on the importation of food into Paris are almost certainly too low, probably due to the very considerable amount of smuggling that was carried on.

The figures show considerable variation, even for the same country. Claassen reported an intake of 99.8 grams digestible protein and 3633 available calories for the urban population of Germany and 146 grams and 5193 calories for the rural population, whereas Lichtenfelt cal-

culated them to be only 104 and 3336 for the country as a whole. Claassen's figures agree fairly well with those of the Eltzbacher commission, but the latter show an increased consumption of wheat at the expense of rye and a lessened meat consumption in the interval of five or six years.

The total energy consumption is over 3400 calories in all countries. The average protein intake is always more than 100 grams. Meat, including fish, poultry and eggs, supplies roughly 20 per cent of the calories and somewhat more than this fraction of the protein; milk and its products, from 13 to 17 per cent of the calories and 14 to 25 per cent of the protein and the cereals, from 35 to 40 per cent of both calories and protein.

The greatest variation is found in the nature of the cereal used. In Great Britain and in France, this is almost exclusively wheat; in this country, maize plays a not inconsiderable rôle; but in Germany, particularly among the rural population, rye is used almost exclusively. (See also pages 365, 376, 377.)

Except in the United States, in Paris and in the German cities, potatoes furnish 10 or 12 per cent of the total energy content and a some-

TABLE IV.—SYNOPSIS

BELGIUM

Date	Authority	Subjects	Number of Studies	Number of Individuals	Scale for Conversion [1]	"Man Equivalents"	Average weight of adult male kilos	Duration days
1853	Engel	Needy families	48					
		Families, income just adequate	51					
		Families, able to save	54					
1891	Engel	Workmen's families: Income less than 280 marks per man per year	44	282	A	193		30
		Income 280–350 marks per man per year	49	315	A	218		30
		Income 350–420 marks per man per year	47	294	A	205		30
		Income over 420 marks per man per year	48	276	A	202		30
		Average	188	1167	A	818		
1908	Slosse & Van der Weyer	Workmen	33	33		33	66.4	6
		Of these, metal-workers (hard work)	8	8		8	70.0	6
		Wood-carvers, shoemakers, etc. (moderate work)	13	13		13	68.4	6
1910	Slosse & Waxweiler through Dunluce & Greenwood	Weavers	156		C			14
		Printers	36		C			14
		Miners	115		C			14
		Quarry workers	49		C			14

[1] See Table II.

what smaller part of the protein. The amount of protein contributed by "other vegetables" is slight in Great Britain and in the United States, is greater in Russia and is considerable in Germany and in Paris, owing to the free use of legumes. The part played by sugar is greatest in the United States and in Great Britain but is considerable in all countries. The consumption in the form of beverages has generally been included in that of the materials used for their preparation but in the reports of Lichtenfelt and of the Eltzbacher commission for Germany and of Gautier for Paris this has been separately calculated and found to amount to from 5 to 14 per cent. It is not surprising, therefore, that the prohibition of the use of alcoholic beverages should, as is claimed for the United States, increase the consumption of sugar and other sweets.

Studies upon Individuals and Groups on Freely Chosen Diets

We now have a general conception of the character of the diet in these countries, considered as units. How is it with the individual? What

OF DIETARY STUDIES

BELGIUM

Composition of Food, per Man, per Day					Percentage Calories from		Percentage Distribution of Protein					
Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories	Protein	Fat	Meat [2]	Milk and products	Wheat	Rye	Potatoes	Others
Calcd.	52.6[3]	17.3[3]	469[3]	2341[3]	10.9[3]	6.9[3]						
"	65.1[3]	29.2[3]	504[3]	2592[3]	10.1[3]	10.5[3]						
"	72.7[3]	39.3[3]	519[3]	2790[3]	10.7[3]	13.1[3]						
"	67.9[3]	56.4[43]	458[3]	2683[3]	10.4[3]	19.2[3]	13.0	5.8	54.3	7.0	14.1	5.9
"	79.5[3]	70.6[3]	497[3]	2985[3]	10.9[3]	22.0[3]	17.7	6.7	41.8	15.5	11.9	6.3
"	97.2[3]	80.6[3]	572[3]	3490[3]	11.4[3]	21.5[3]	18.5	9.0	46.7	8.4	10.2	7.2
"	108[3]	93.1[3]	571[3]	3646[3]	12.1[3]	23.7[3]	22.4	10.1	46.8	2.5	9.0	9.2
"	85.9[3]	74.9[3]	521[3]	3179[3]	11.1[3]	21.8[3]						
Anal.	105[5]	100	393	2932	14.7	31.6						
"	117	115	410	3110	15.4	34.3						
"	100	107	381	2815	14.6	35.4						
Calcd.	(80.6)[4]	(86.9)[4]	(529)[4]	(3336)[4]	9.9	24.2						
"	(94.9)[4]	(103)[4]	(586)[4]	(3817)[4]	10.2	25.1						
"	(77.2)[4]	(127)[4]	(497)[4]	(3604)[4]	8.8	32.8						
"	(86.2)[4]	(130)[4]	(658)[4]	(4314)[4]	8.2	28.0						

[2] Includes fish, poultry and eggs.
[3] All of the values for food consumption reported by Engel are too low since not all, but only the principal, foods were included.
[4] Figures in parentheses represent digestible nutrients.
[5] "Digestible" protein 0.91 to 2.02 gm. per kilo per day, average 1,375. The man who had only 0.91 gm. protein per kilo lost 3.48 gm. nitrogen per day

TABLE IV.—SYNOPSIS OF

Date	Authority	Subjects	Number of studies	Number of individuals	Scale of Conversion	Man equivalent	Average weight of adult male kilos	Duration days	Composition of Food, per Man per Day: Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories
Denmark													
1910	Heiberg and Jensen	Laborers' families in Copenhagen..	27		F (?)	83.7			Calcd.	107	105	493	3351
		In other towns...	23		"	76.6			"	101	90	464	3153
		In islands ..... / In Jutland	201		"	589			"	109 / 119	111 / 103	516 / 550	3595 / 3701
		Average.....	251			749			"	107	105	493	3450
1912	Hindhede..	Author's family...	1	10		7			"	76	103		3418
Finland													
1904	Sundstrom	Students								160	200	391	4126
		University.....	1	100		100	67.6	14	Calcd.	157[7]	191[7]	380[7]	3984[7]
		Agric. School, men	1	24		24	61.8	14	"	226[7]	119[7]	685[7]	4836[7]
		" " women	1	9	Not con	verted	*56.8*	14	"	*150*[7,9]	*92*[7,9]	*496*[7,9]	*3508*[7,9]
		Families of city workmen.....	12	40	C	30.8		14	"	139	130	455	3643
1907	Sundstrom	Farmers, etc., men	17	17			67	7	Anal.	136	83	580	3705[9]
		" women	25	25			*59*	7	"	*91*[9]	*51*[9]	*360*[9]	*2326*[9,10]
		" " disregard 6 lowest	19	19			*59*	7	"	*94*[9]	*54*[9]	*392*[9]	*2452*[9,10]
1907	Sundstrom	Households of farmers, etc....	80	559	H	393		7	Calcd.	177	104	688	4516[10]
France													
1906	Gautier ..	Family of farm laborer in south of France.....	2	14	(fn.[11])	12		385	Calcd.	149	79	830	4745

[6] Includes oleomargarine. [7] Corrected for waste. [8] Includes other vegetables. [9] Figures in italics refer to food consumption per woman, not per man equivalent. [10] Sundstrom gives other figures but he used other factors for energy values of food. [11] Gautier calculated food consumption of 2 women and child of 7 as equivalent to that of one man.

variation is there among individuals and what are the factors responsible for such variation?

There have been many observations published on the food consumption of individuals and of groups living on their customary diet, which is sometimes called a "freely chosen diet." In reality there is no such thing. Man's choice is limited by his geographic and economic situation, to say nothing of such things as food habits and prejudices acquired early in life. Just as was his primitive ancestor, though to a lesser degree, modern man is limited in his choice by his environment.

Among the earliest reports that are sufficiently accurate to be of any considerable value are those of Liebig on the food of Bavarian woodchoppers. Similar studies were made by Playfair, by Meinert, by Moleschott and by others but the greatest impetus to the study of the food habits of the people appears to be due to the work of Voit. Basing his opinion upon the results of previous investigators and upon the actual food con-

DIETARY STUDIES—*Continued*

DENMARK

Percentage Calories from		Percentage of Distribution of Protein					Percentage Distribution of Calories						
Protein	Fat	Meat[2]	Milk and Products	Cereals	Potatoes	Other vegetables	Meat[2]	Milk and Products	Cereals	Potatoes	Other vegetables	Sugars	Others
13.1	29.1												
13.1	26.6												
12.4	28.7												
13.2	25.8												
12.7	28.3												
9.1	28.0	5.7	34.8	46.2	12.7[8]	0.5	1.6	31.7[6]	36.9	12.7		14.1	2.7

FINLAND

15.9	45.1	43.4	30.9	19.9	2.3	2.5	21.9	39.5	24.1	4.3	1.6	7.3	
16.2[7]	44.6												
19.1[7]	22.9	17.3	43.2	29.6	5.1	4.8	13.5	28.8	44.0	10.2	3.5		
17.5[9]	*24.4*	19.0	38.8	32.2	2.9	7.1	13.1	27.0	43.9	13.9	2.2		
15.7	33.2	28.1	38.0	28.1	1.7	4.0	11.0	39.0	33.7	7.4	1.7	7.2	
15.0	21.6	19.0	36.0	37.0	8.0		9.0	28.0	50.0	13.0			
16.0[9]	*20.8*[9]												
15.7[9]	*20.5*												
16.1	21.4	15.0	35.0	41.0	7.0	2.0	10.0	27.0	48.0	11.1		3	

FRANCE

15.5	12.9												

sumption of men of average weight, 70 kilos, engaged in moderate work in the city of Munich, he concluded that a normal diet for such a man should contain 118 grams of protein, 56 grams of fat and 500 grams of carbohydrate. Substitution of as much as 150 grams of the carbohydrate by an isodynamic amount of fat was considered desirable. This is known as Voit's standard. As Dunluce and Greenwood say, "It has enjoyed a vogue which is not so much due to the number or accuracy of the laboratory experiments carried out by Voit as to this investigator's high and well-deserved reputation." However, the necessity of so large an amount of protein has been vigorously denied and as vigorously affirmed. The question will be considered later.

Some of the evidence is contained in Table IV, which gives a summary of some of the results obtained in what seem to be some of the more important studies of people on their accustomed diets made since Voit's time. Most of these were made on the poorer classes of the

TABLE IV.—SYNOPSIS OF

Date	Authority	Subjects	Number of studies	Number of individuals	Scale of conversion	Man equivalent	Average weight of adult male kilos	Duration days	Composition of Food per Man per Day: Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories
Germany													
1880–1892	Demuth...	Pensioners, etc., light work.....	3	3	Calcd. to 70 kilos body w't.				Calcd.	103	50	546	3130
		City laborers.....	2	15	Calcd. to 70 kilos body w't.				"	131	67	545	3472
		Farm laborer.....	1	1	Calcd. to 70 kilos body w't.				"	137	89	590	3811
		Families of above, etc.	20	78	Calcd. to 70 kilos body w't.					99	57	597	3400
1890	V. Rechenberg.....	Families of hand-weavers, very poor	28				57[12]	7	"	65[12]	49[12]	485[12]	2703[12]
1899	Ranke.....	Physician (self), Jan. and Feb....	1	1			73	30	"	138	162	351	3512
	Ranke.....	Physician (self), July and Aug...	1	1						135[13]	162[13]	372[13]	3588[13]
1902	Neumann..	Laboratory investigator (self)....	1	1			67.5	305	"	66	84	230	2309
		Laboratory investigator (self)....	1	1			66	15	Anal.	77	156	221	2659
		Laboratory investigator (self)....	1	1			72	321	Calcd.	76	109	169	2068
1895	Atwater...	Bavarian mechanics.....	17						"	134	63	491	3150
		" farmers	5						"	137	55	545	3295
		" brewery laborers	5							149	61	755	4275
1910	Claassen...	Peasant families, Rhine valley....	30		[17]					109	146	669	4537
Greenland													
1857	Krogh, A. & M..	Eskimos	65							282[A]			2604[A]

[12] Per adult individual. [13] See text, page 389. [14] 12.7% protein in beer. [15] 1.4% protein in beer. [16] Legumes furnished 4.5% of the protein and 1.8% of the calories. [17] It is not evident just what factors were used, but they were apparently lower than any of these in Table II. [A] Not all food included.

population and many of them were undertaken to ascertain whether or not a condition of undernutrition obtained. For this reason, it is probable that the values reported are minimal rather than optimal. In order to facilitate comparison, the results have been grouped by countries and within each group have been arranged chronologically, unless other considerations made some other arrangement appear preferable.[1]

[1] There is much valuable material for the student of nutrition in the series of family monographs published by Le Play under the title "Les ouvriers européens" and continued by the Société internationale des études pratiques d'economie sociale as "Ouvriers des deux mondes." These are a series of complete studies of families in many parts of the world and include the amount paid for food, in money, kind or labor, and the amount and nature of the food secured. Unfortunately, the character of the food is not always sufficiently well-defined to permit of accurate calculation. A similar criticism applies to the reports of the Board of Trade of Great Britain on working-class conditions in Great Britain, Belgium, France, Germany and the United States.

DIETARY STUDIES—*Continued*

GERMANY

Percentage Calories from		Percentage Distribution of Protein						Percentage Distribution of Calories						
Protein	Fat	Meat [2]	Milk and Products	Cereals	Potatoes	Other vegetables	Others	Meat [2]	Milk and Products	Cereals	Potatoes	Other vegetables	Sugars	Others
14.3	14.7													
15.5	17.9													
15.7	22.7													
12.0	15.7													
9.9	17.0							1.1	12.0	61.7	18.4			
16.1	42.8													
15.4	41.8													
11.7	33.6	35.9	27.4	22.5	1.6		12.7[14]							
11.8	54.6													
15.1	48.9	47.0	27.6	19.2	1.9		4.2[15]							
17.5	18.5													
17.0	15.5													
14.3	13.3													
11.1	30.0	12.8	21.7	41.1	11.2	12.6[16]	0.6	14.8	18.8	42.0	15.6	5.0[16]	1.9	1.1

GREENLAND

44	48													

The first column gives the date of the study if that is available, if not that of the publication and the next, the name of the author or other authority for the data. The succeeding columns give, in order, some idea of the social and economic status of the subjects, the number of studies, the total number of individuals, the scale of conversion to "man equivalents," the number of these, the average weight of an adult male and the average duration of the studies. These fall into two classes, according as the data for the composition of the food were obtained by actual

These include the results of questionnaires on family budgets. Some of the additional difficulties in drawing conclusions from some of the calculations that have been made from some of the Board of Trade data are discussed in footnote 21 to Table IV, p. 378.

However, cursory examination of the French monographs and of the reports of the Board of Trade indicates that more detailed consideration would only corroborate the conclusions indicated by the data presented in this chapter.

TABLE IV.—SYNOPSIS OF

GREAT BRITAIN

Date	Authority	Subjects	Number of		Scale for conversion	"Man equivalents"	Average weight of adult male kilos	Duration days
			Studies	Individuals				
1900	Paton, Dunlop and Inglis	Families in Edinburgh:						
		Income less than 20s.; av. 17s., 4d	5	32	C	18		7
		Av. income 22s., 2d	5	30	"	17.1		7
		Av. income 39 s	4	34	"	21.4		7
		Typical, av. income 25s., 10d	9	50	"	34.4		7
1901	Rowntree, data recaled. by Dunluce and Greenwood	Families in York:						
		Av. income 18s., 11d. (all under 26s.)	16	87	I	58.5		70
		Income over 26s	3	17	I	12		19
		Servant keeping	6	39	I	30		9
1904	Board of Trade; calcns. by Dunluce and Greenwood and by Greenwald	Families of workmen in cities:						
		Income under 25s.; av. 21s., 4½d.		261	See Note [21]			
		Income 25–30 s.; av. 26s., 11¾d.		289				
		Income 30–35s.; av. 31s., 11¼d.		416				
		Income 35–40s.; av. 36s., 6¼d.		382				
		Income 40s. or more, av. 52s., ½d.		596				
1911	Cameron	Edinburgh students: men	4	149		149		7
		Edinburgh students: women	1	30	0.8	24		7
1911–12	Lindsay	Glasgow families:						
		Income under 20s, average 18s, 14d	5	29	C	18		7–14
		Income 20–25s, average 23s, 10d	10	63	C	39.2		7–14
		Income 27–31s	3	20	C	11.2		7–14
1916	Ferguson	Glasgow families:						
		Average income 27.2s	6		C			
		Average income 31.3s	4		C			7
1917	Ferguson	Average income 28.4s	6		C			7
		Average income 50.6s	4		C			7
1903	Dunluce and Greenwood	British Agricultural Laborers			see note [21]			
		Northern Counties						
		Midland Counties						
		Eastern Counties						
		Southern and Southeastern Counties						

[18] Includes 2.2% from peas.
[19] Includes 13.6% from sugar.
[20] Figures underlined refer to distribution of calories, not protein.
[21] The average number of children in the families in the different groups was 3.1, 3.3, 3.2, 3.4, 4.4 and 3.6, respectively. In their calculations, Dunluce and Greenwood used the value 0.51 to convert the number of children into "man equivalents." But

DIETARY STUDIES — *Continued*

GREAT BRITAIN

COMPOSITION OF FOOD PER MAN PER DAY					PERCENTAGE CALORIES FROM		PERCENTAGE DISTRIBUTION OF PROTEIN					
Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield grams	Protein	Fat	Meat [2]	Milk and products	Cereals	Potatoes	Others	
Calcd.	93	69	396	2607	14.6	23.6	27.1	10.6	53.2	9.0		
"	103	82	480	3133	13.5	24.4	34.1	7.0	54.7	4.1		
"	115	92	529	3531	13 4	24.3	31.9	10.0	53.0	5.0		
"	108	88	479	3228	13.7	25.5	30.3	10.2	53.0	3.5	3.3[18]	
							16.3[2]	12.8[2]	50.2[2]	4.6[2]	16.3[19-20]	
"	82	88	450	3000	11.2	27.3			49.4[20]			146 grams meat and 85 grams sugar per day
"	117	130	589	4102	11.6	29.7			45.3[2]			227 grams meat and 88 grams sugar per day
"	112	161	511	4052	11.3	37.0			29.7[2]			280 grams meat and 113 grams sugar per day
"	86	59	536	3094	11.4	17.6			61.3[2]			101 grams meat and 73 grams sugar per day
"	92	71	565	3348	11.2	19.6			55.2[20]			117 grams meat and 85 grams sugar per day
"	99	82	588	3581	11.3	21.3			55.5[2]			142 grams meat and 93 grams sugar per day
"	98	86	582	3589	11.0	22.5			51.0[2]			146 grams meat and 98 grams sugar per day
"	108	100	644	4013	11.0	23.1			53 3[2]			154 grams meat and 110 grams sugar per day
"	140	138	516	3976	14.4	32.3						
"	162	139	495	3990	16.6	32.4						
"	98	76	385	2689	14.9	26.4	39.5	8.4	46.9	3.8	1.5	
"	118	86	531	3457	13.9	23.1	29.1	9.9	51.8	4.1	3.6	
"	118	98	506	3648	13.9	26.3	31.4	10.8	50.2	3.6	3.9	
"	96	96	467	3198	12.3	27.9						
"	98	88	439	3017	13.4	27.2						
"	93	72	462	2949	13.0	22.8						
"	112	89	498	3331	13.8	24.8						
"	88	113	547	3654	9.9	27.8						
"	88	90	537	3698	10.6	24.6						
"	92	83	597	3598	10.5	21.5						
"	96	84	600	3634	10.8	24.6						

in the families with the larger incomes it is probable that some of the family income came from the earnings of some of the children. These children would be older than the average and would eat more. Even if this effect be disregarded, the families with smaller income would be likely to those most recently established, with the younger children, whose food consumption would be lower than the average. The effect of income upon the amount and character of the food consumed is, therefore, probably exaggerated in these figures.

TABLE IV.—SYNOPSIS OF

INDIA

Date	Authority	Subjects	Number of Studies	Number of Individuals	Scale for Conversion	"Man equivalents"	Average weight of adult male kilos	Duration days
1908	McCay (1908)	Bengali students, ration scale....	1				54	
		Anglo-Indian and Eurasian students, same college.....	1					
1912	McCay (1912)	Bengalese cultivators.....						
		" middle classes, not above indigence.....					Approx. 50	
		Bengalese middle classes, above indigence.....						
		Thibetans, etc., rickshaw men..						
		Sikhs, young men.....						

ITALY

Date	Authority	Subjects	Number of Studies	Number of Individuals	Scale of conversion	"Man equivalents"	Average weight of adult male	Duration days
1886	Lichtenfelt(1903)	Workers in food industries....	5					
		Textile workers.....	9					
		Laborers.....	7					
		Miners.....						
1894	Memmo.....	Men at moderate work, Rome, ordinary diet.....	3	3			60.7	7
		Native of chestnut-eating district, chestnut diet, easy work.....	1	1			59.1	7
		Acorn diet, very light work...	1	1			65.5	
1893	Manfredi.....	Poor men, Naples, cobblers....	2	2			51	5
		" man, " mason.....	1	1			55	5
		" " " carpenter..	1	1			62	7
1906	Albertoni and Rossi	Peasants of the Abruzzi, men	7	7			60.4	5
		" " " " women	5	5	Not converted		*50.8*[9]	*5*

JAVA

1892	Eijkman (1893)	Malays, Laboratory servants....	4	4			47.5	4.5
		" medical student.....	1	1			58.1	5
		Europeans in Java, physicians, etc.....	11	7			65.4	4

[9] Figures in italics refer to food consumption per woman, not per "man equivalent."

DIETARY STUDIES — *Continued*

INDIA

Composition of Food per Man per Day					Percentage Calories from		Percentage Distribution of Protein					
Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories	Protein	Fat	Meat [2]	Milk and products	Rice	Other cereals	Legumes	Other vegetables
Calcd.	67	72	549	3190	8.6	20.8	13.9		30.5	19.9	26.9	8.7
"	95	56	467	2822	13.8	18.5	41.6	4.4	13.4	26.8	12.9	2.1
"	52	25	475	2390	8.9	9.8	9.7		87.3		5.7	2.2
"	50	50	400	2310	8.9	20.5	10.1	7.5	72.5		6.9	2.3
"	70	90	300	2350	12	36	14.4+	10.7	19.4	41.2	4.3	1.6
"	175–200			6300+								
"	125–130			3750-4000[22]								

[22] Includes 16 oz. milk and 4 oz. meat per day.

ITALY

Composition of Food per Man per Day					Percentage Calories from		
Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories	Protein	Fat	
Calculated	143	31	713	3808	15.4	7.6	
"	128	29	662	3470	15.1	7.8	
"	168	48	909	4866	14.2	9.2	
"	227	62	932	5326	17.5	10.8	
Analyzed	106	30	495	2745	15.8	10.2	87 grams digestible protein and 2563 available calories
"	59	19	464	2521	9.6	7.0	44.4 grams digestible protein and 2171 available calories
"	124	63	252	2120	24.0	27.4	98 grams digestible protein and 1892 available calories
"	75	38	379	2208	13.9	15.4	
"	71	29	391	2155	13.4	12.3	
"	94	56	475	2852	13.5	18.3	
"	73	53	450	2746	10.9	18.1	52.9 grams digestible protein and 2480 available calories
"	*60*[9]	*46*[9]	*348*[9]	*2204*[9]	*11.2*[9]	*19.4*[9]	42.7 grams digestible protein and 2004 available calories

JAVA

Anal.	70	29	482	3254	8.9	8.3						
"	96	64	426	2731	14	22						
"	98	92	262	2553	16	34						

TABLE IV.—SYNOPSIS OF

JAPAN

Date	Authority	Subjects	Number of Studies	Number of Individuals	Scale for conversion	"Man equivalents"	Average weight of adult male	Duration days
1886	Eijkman (through Oshima)	Prisoners, no work	1	20+			47.6	
		" light work	1	20+			48.0	
		" hard work	1					
1889	Nagase (Oshima)	Military colonist in Formosa	1	1			59	7
1890	Tsuboi (Oshima)	Jinrickshaw man	1	1			62.4	4
1909	Inaba	Farmers, rice diet	7					
		" barley-rice diet	7					
		" average of all	14					
1910	Yukawa	Celibate monks, young, no work	8	8			44.5	7
	"	Celibate monks, light work	1	1			52.1	7
	"	Celibate monks, old, no work	3	3			51.8	7
1911	Hinhede (1920)	Diet list of Japanese pavilion, Dresden, 1911, hard work	1	7				7
		light work	1	5				7
1919	Kobu and Sokamoto	Workmen	4	2				32

RUSSIA

Date	Authority	Subjects	Number of Studies	Number of Individuals
1889	Erismann (1889)	Factory workers	50	1670
1904	Smolensky	Factory workers, ordinary diet	3	
		" " fast days	3	
		Peasants, Government Moscow, poor	2	
		" " " well-to-do	2	
		Laborers, Cronstadt docks, ordinary diet	1	
		" " " fast days	1	
		Laborers and mechanics, Cronstadt, wages 18–24 rubles per month, 5 spent for food	1	
		Ditto, 24–28 rubles, 7.5 spent for food	1	
		Ditto, 30–48 rubles, 13.5 spent for food	1	
		Fishers at mouth of Volga, men	1	
		" " " " " women	1	
		Peasants, 2 districts, men	1	
		" 2 " (same), women	1	
		Average of all reported by Smolensky	94	

* Figures in italics refer to food consumption of women not "man equivalents."

DIETARY STUDIES—*Continued*

JAPAN

Composition of Food per Man per Day					Percentage Calories		Percentage of Distribution of Protein				
Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories	As Protein	As fat	Meat[2]	Cereals	Legumes	Other vegetables	
Anal.	48	6.8	372	1782	11.0	8.6					
"	57	7.6	458	2178	11.7	3.2					
"	75	9.8	630	2975	10.3	2.9					
"	59	7.7	594	2752	8.9	2.3					
Calcd.	158	25.6	1031	5113	12.7	4.7					
"	78	16.9	530	2676	11.9	5.9					
"	126	31.6	663	3529	14.6	8.3					
"	102	24.3	597	3091	13.5	7.2					
Anal.	57	14.6	345	1804	12.9	7.5					38 grams digestible protein and 1651 available calories
"	87	21.2	531	2719	13.1	7.3					63 grams digestible protein and 2547 available calories
"	60	12.3	347	2020	12.3	5.7					41 grams digestible protein and 1872 available calories
Calcd.	126	31.5		3536	14.6	8.3	5	63	32		
"	81	18.6		2770	12.0	6.2	7.5	76	7	9.5	
"	96	18.9	766	3766	10.4	4.7					

RUSSIA

Composition of Food per Man per Day					Percentage Calories from	
Calculated	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories	Protein	Fat
Calculated	132	80	583	3676	14.7	20.2
	133		565	3507	15.5	18.8
	121	71	603	3706	13.4	20.0
	109	80	542	2935	15.2	9 2
	146	29	669	3784	15.8	11.8
	220	48	931	5603	16.1	15.7
	216	95	1040	6033	14.7	14.6
	123	43	563	3207	15.7	12.3
	122	52	419	2704	18.5	18.0
	146	140	460	3785	15.8	34.4
	303	71	462	3797	32.5	17.3
	219[9]	*43*[9]	*463*[9]	*3194*[9]	*28.1*[9]	*12.5*[9]
	138	39	560	3223	17.5	11.2
	122[9]	*31*[9]	*525*[9]	*2842*[9]	*17.6*[9]	*10.0*[9]
	149	57		4040	15.1	13.1

TABLE IV.—SYNOPSIS OF

Date	Authority	Subjects	Number of		Average weight of adult male kilos	Duration days	Composition of Food per Man per Day				
			Studies	Individual			Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories
Sweden											
1887	Hultgren and Landergren (1889)	University students	5	5	68	10.4	Calcd.	128	115	300	3034
1887	Hultgren and Landergren (1889)	University professor	1	1	96	8	"	137	113	345	3205
1887–8	Hultgren and Landergren (1891)	Workingmen	11	9	67	7.5	"	159	94	610	4023
1893–8	Englund (Tigerstedt, 1900)	Lumbermen in north of Sweden: "Rivermen"	17	17	64.4	22	"	124	214	424	4239
		Choppers, etc	96	96	67.3	65	"	140	284	732	6214
		Of these latter	1	1	72	56	"	181	415	1145	9292
		Lumbermen, etc., groups		119	68		"	130	271	696	5905
		Of these a group of 2 men	1	2	69		"	152	523	720	8439
Switzerland											
1912	Gigon	Workmen	8	8	68.9	7	Anal.	107	93	402	3181

[23] Beer. [23a] Legumes.

analysis of samples of the material used in these studies or were obtained by calculation from published analyses of similar food materials, with or without occasional supplementary analyses by the author. The figures in the following columns represent the daily intake per man (if in italics, per woman) of protein, fat, and carbohydrate. Then follow the total energy intake, the fractions of this contributed by protein and by fat, the contributions to total protein and total energy content made by the different classes of food materials and other data that appeared to be of interest.

Some of the figures have been taken from the original publications, some have been obtained through other authors, as indicated, and some have been calculated by the writer. Many of the publications cited contain data that permit of calculations to fill many of the vacant spaces in the table but the labor of such calculations is onerous, and seems to be out of proportion to the value of the results to be expected.

From the material presented in previous chapters, it is evident that the food consumed must supply energy for the following demands: 1. the basal metabolism, 2. the increase in metabolism due to the ingestion of food, 3. the increase in metabolism due to muscular work, 4. the mainte-

DIETARY STUDIES—*Continued*

SWEDEN

Percentage Calories from		Percentage Distribution of Protein						Percentage Distribution of Calories					
Protein	Fat	Meat [2]	Milk and Products	Cereals	Potatoes	Other vegetables	Others	Meat [2]	Milk and products	Cereals	Potatoes	Other vegetables	Others
17.3	35.3	47.7	16.8	15.3									
17.5	32.8	52.6	10.6	20.6									
16.2	21.6	28.1	21.4	37.8	5.9	4.6	3.1[22]	14.7	18.8	46.9	10.4	4.2	2.8[23]
12.0	47.0	31.4	23.5	42.8		0.1[23a]							
9.3	42.4	28.2	2.9	60.3	5.2	2.9[23a]							
8.0	41.5	26.2		58.2	11.1	4.5[23a]							
9.7	42.6												
8.0	41.5	45.4		54.6									

SWITZERLAND

13.8	27.2												

nance of body temperature. Variations in the amounts of energy required for these purposes mean variations in the amount of food required and, presumably, in the amount consumed. This we shall find to be the case.

The many variables involved make direct comparison of the tabulated figures difficult but by considering only one at a time, fairly regular relations appear.

Influence of Climate and Season upon Food Consumption.—It is a generally accepted belief that less food is required in summer than in winter and less in the tropics than in temperate climates. But there are very few accurate observations and such as there are do not support this belief.

In a study of the rations consumed by a battalion of French soldiers, Perrier found an apparently regular change with the season. (Table V.) But these soldiers were fresh recruits in October and Perrier ascribed the large consumption of food in October and November to this fact. The peak came in November, the consumption of food being then 100 calories greater than in the following January and February. When the men were at camp, June 22 to July 11, the new mode of life and, probably, the in-

TABLE IV.—SYNOPSIS OF

UNITED STATES

Date	Authority	Subjects	Number of		Scale for conversion	"Man equivalents"	Average weight of adult male kilos	Duration	Composition of Food per Man per Day				
			Studies	Individuals				days	Calculated or analyzed	Protein grams	Fat grams	Carbohydrate grams	Energy yield calories
1920	Pearl	Selected studies in American families, with average annual income of each group											
		Mother wage earner $640	8		J	212[24]			Calcd.	105	65	472	2895
		Garm'tmakers $724	7		J	168[24]			"	109	81	495	3145
		Laborers.....$1497	6		J	305[24]			"	94	102	479	3210
		Retired.....$1647	5		J	136[24]			"	81	121	420	3095
		Clerks (office)$1934	11		J	225[24]			"	92	120	419	3125
		Mechanics...$2133	8		J	259[24]			"	97	113	460	3245
		Teachers.....$2150	32		J	620[24]			"	88	125	430	3195
		Profess'l men $2208	17		J	438[24]			"	99	148	438	3480
		Engineers (professional).....$2253	5		J	97[24]			"	85	128	395	3070
		Salesmen....$2527	5		J	121[24]			"	90	111	405	2980
		Farmers.....	12		J	384[24]			"	102	131	506	3640
		Average.....	116		J				"	95	113	447	3185
1903	Atwater (1903)	Farmers.....	14		G				Calcd.	108	136	493	3767
		Athletes.....	23		G				"	181	194	506	4617
		Business men, students.....	41		G				"	124	142	451	3678
1904	Woods and Mansfield	Maine lumbermen, "Chopping and yarding".....	2	47 or 77			75.8	11	Calcd.	206	387	963	8140
		Average of all operations.....	5	174 or 200			73.1	9.4	"	182	337	812	6995
1917–8	Murlin...	U. S. soldiers in training camps (supplied)[26 27]...	427					7	Calcd.	131	134	516	3899
		Consumed.....	427					7	"	122	123	485	3633
		Consumed plus canteen purchases	427					7	"	127	136	545	3998
		Of these	213					7	"	138	133	527	3963
		(consumed)[27]						7	"	129	121	496	3687
1917	Benedict, Miles and Roth	Students.....	12	12			66.0	3	Anal.	97			3097
1896–7	Atwater and Bryant [B]	Workmen's families, New York City, children of normal weight...	10	No. of children in family 3.7	C			10	Calcd.	101	124	382	3175
		Children below normal weight...	11	4.3	C			10	"	92	95	349	2693
1901–4	Wait.... [B]	Families, eastern Tennessee children of normal weight.....	28	2.8	C			14	"	77			3601
		Children below normal weight...	10	2.6	C			14	"	75			3304

[24] "Man equivalents" multiplied by number of days.

[26] Army rations are not generally considered a freely chosen diet but under the system in use at the training camps during the period of these studies, the rations were, within the limits imposed by geographic and economic considerations, practically the "free choice" of the mess sergeants. They were supplemented by individual purchases at the regimental exchange. Both sources of food were included in these studies.

[27] Supplied and consumed at army mess. Canteen purchases not included.

[B] See page 416.

DIETARY STUDIES—*Continued*

UNITED STATES

PERCENTAGE CALORIES FROM		PERCENTAGE DISTRIBUTION OF PROTEIN					PERCENTAGE DISTRIBUTION OF CALORIES					
Protein	Fat	Meat [2]	Milk and products	Cereals	Vegetables	Fruit	Meat [2]	Milk and products	Cereals	Vegetables	Sugars	Fruit
15	21											
14	24											
12	30											
11	37											
12	36											
12	32											
11	36											
12	40											
11	39											
12	34											
11	33											
12	33											
11	34											
15	39											
13	37											
10	44											
11	45	44.8	0.3	26.3	27.8[25]	0.6	43.1	4.2	24.3[25]	13.8[25]	11.1	3.5
14	32											
14	32											
13	31											
14	31											
14	32	46.9	4.0	26.4	4.4	5.3	12.3	28.7	3.0	30.3	5 7	2.9	12.3	16.5
13														
13	36	47 2	11.4	31.8	Vegetables 8.3	Fruit 1.2		27.9	13.6	39.6	Vegetables 7.7	Fruit 0.8	10.3	
14	32	46.6	8.3	34.3	10.8	0.2		25.8	13.6	38.2	9.6	1.1	11.6	
8.8		11.7	7.3	71.4	8.4	0.3		21.1	6.2	60.4	6.6	1.3	4.5	
9.3		12.7	6.8	64.6	14.6	0.2		19.7	5.9	57.4	7.2	6.9	1.8	

[25] Chiefly beans

creased exercise, led to a consumption of 4065 calories, which far exceeded the maximum of the previous winter. During the year, the men gained an average of 742 grams in weight. It is probable that most of this gain

occurred in the first few months and thus accounts for the large food consumption at that time.

TABLE V.—FOOD CONSUMPTION OF SOLDIERS IN DIFFERENT MONTHS OF THE YEAR

Subjects \ Month	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov	Dec.
Battalion French recruits 1908–1909	3682[1]	3789	3765	3681	3695	3670	3648	3599	3435[2]	4065[3]	3458[4]				
Men in U. S. training camps 1917–1918 No. of studies	3	19	36	37	30	42	77	30	20	13	14	8	13	7	5
Food consumption	3606	3706	3819	3827	3864	3894	3545	3514	3517	3609	3658	3487	3727	3918	4145

[1] October 10 to 31.
[2] June 1 to 17.
[3] This period at camp, June 22 to July 11.
[4] July 12 to Aug. 12.

During 1917 and 1918, a series of nutritional surveys were made in the training camps of the United States Army. (See Table IV.) Although they were not made upon the same men throughout the year, the observations were so numerous and each made with so large a number of men, probably over 200, as to furnish useful averages for the present purpose. When the energy content, in calories, of the food consumed per man per day is calculated for the different months of the year, as in Table V, certain seasonal changes become evident. Beginning in October, 1917, the figures showed a gradual increase in food consumption until it reached 3894 calories in March, falling to 3545 in April. This level was continued in May, June and July. In August, there was a slight rise but in September there was a return to the summer level, after which there was a rise to December, 1918, at which time the observations ended. The peak of the previous years was passed in November and the food consumption in October, November and December was, respectively, 121, 212 and 326 calories greater than in the corresponding months of the previous year. Attempts to correlate the curve of food consumption with variations in local temperature, wind velocity, humidity, etc., were not successful. It would seem more likely that the higher consumption of food in the winter was due to the greater muscular activity of the men. There is, moreover, another factor of possibly even greater importance. Practically all the men in training gained weight. If this gain did not occur in summer or was then much smaller than in winter, this difference alone would account for the differences in food consumption. The effect of the armistice in modifying the attitude of the men in regard to the conservation of food may help to account for the larger food consumption during the last two months.

According to Eijkman(*b*) (1897), the basal metabolism of Europeans in Java was not lower than in Europe and Dutch physicians there ate

as much food as men of similar occupation in Holland. Similarly, Ranke, in Munich, found that he required as much food *to maintain his body weight* in summer as he did in winter.

The explanation of this uniformity of food consumption over a wide range of external temperatures appears quite obvious. Except under very unusual circumstances, man selects his clothing so as to keep the temperature of most of the body surface at about 30° C. If the customary activities of the individual involve a heat production which is too great to be dissipated with maintenance of surface temperature at 30°, the individual may, and generally does, diminish his food consumption but only at the cost of loss of body substance or ability to do work. Thus Ranke, in the experiment above referred to, found that, of free choice, he would have consumed 400 calories less per day during the summer but that he then lost weight which, for the purpose of the experiment, was to be kept constant. He accordingly ate enough to maintain his body weight but experienced increasing discomfort until, at the end of the month, there was a definite gastro-intestinal disturbance and, apparently, an increased susceptibility to infection. It is important to remember, in this connection, that the average temperature of the room in which Ranke spent most of his time was 21.9° C. in summer and 18.9° C. in winter. The humidity is not stated but was probably lower in winter than in summer, so that the cooling effect of the air was greater in winter than in summer. Moreover, when indoors, Ranke wore the same clothing in summer as in winter, so that it seems quite likely that the dissipation of heat was interfered with and that this led to the disturbances he noted.

If external conditions, such as temperature and humidity, do not permit the removal of the heat produced in ordinary metabolism, the temperature of the body is raised, the basal metabolism is raised and may thus be even greater in warm weather than in moderate (Young).

It is quite possible that the inability to maintain a high metabolism in warm weather and in the tropics is responsible for the indolence and lack of energy displayed by man under those conditions.

With very low external temperatures, on the other hand, the heat produced in metabolism may not be sufficient to cover the heat loss, even though this be reduced to a minimum by means of much clothing. The feeling of cold is experienced and muscular activity is increased (shivering), with consequent increase in the production of heat. With short periods of exposure, shivering may not appear and, in such cases, as found by Eijkman(*d*) (1897), metabolism is the same at from 6° to 12° C. as at 24.5° C. though the clothing be light and the subjects complain of cold at the lower temperature. There may be some direct stimulating effect of cold upon metabolism (see discussion in Tigerstedt(*h*), 1919, Vol. I, page 168), but such action must, ordinarily, play a very inconsiderable part.

The effects of season and of climate upon the energy content of the food may therefore be neglected except as they may affect the body weight or conduce to, or be unfavorable to, muscular activity.

Relation of Body Weight to Food Consumption.—The basal metabolism is roughly proportional to the body weight (Harris and Benedict) and, consequently, so is the energy content of the quota of food needed to satisfy this requirement.

The increase in metabolism due to the ingestion of food depends upon the amount and nature of the food consumed, the nature of the individual and upon other factors which seem to make it vary from time to time in the same individual with the same kind of food (Benedict and Carpenter). But this constitutes only a small part of the total metabolism and may therefore also be considered as proportional to the body weight.

The same relation holds for the amount of energy required to move the body about. That required to supply energy for external work varies with the nature and amount of the work to be performed and with the muscular efficiency of the individual. But it is probable that, as a rule, in occupations involving much muscular work, the individual weighing considerably less than 70 kilos (154 pounds) will do less than one of that, or slightly greater, weight.

Except in the case of individuals of unusual body form, the total metabolism and, consequently, the food requirements of adults leading about the same kind of life may, therefore, be expected to be proportional to the body weight. With much greater body weights, the proportionality can no longer be expected to hold for an ever increasing part of the weight is contributed by the comparatively inactive adipose tissue. And this, in truth, is usually found to be the case. Selecting from Table IV, groups within which other factors may be considered to be relatively constant and consulting the original publications for the data, we obtain the following figures for the food consumption in calories per kilo of body weight:

Demuth, 3 pensioners, light work, 47, 46, 41	Average	45
Yukawa, Japanese celibate monks, young, no work, 36.5, 50.0, 41.6, 39.0, 36.3, 40.1, 23.9, 34.1,	"	37.7
old, no work, 37.9, 35.6, 35.0	"	36.2
Eijkman (1893), European physicians, etc., in Java, 32.2, 38.7, 32.0, 44.9, 36.6, 30.5, 31.5, 33.3	"	35.0
Eijkman, Malay laboratory servants, 49.6, 41.7, 55.4, 55.8	"	50.6
Hultgren and Landergren, Swedish students, 40.9, 48.4, 44.2, 38.1, 46.8	"	43.7

Within any one group, the energy content of the food consumed is almost as proportional to the body weight as the basal metabolism is found to be (Harris and Benedict). The factors, such as varying body form, differences in activity of endocrin glands, that account for the latter will, probably, also explain the latter. The effect of variations in body weight in the *same* individual upon the amount of food required to maintain a particular body weight will be considered later. (Page 414.)

Influence of Work.—Reference has been made to the variations in energy requirement with differences in the amount of muscular work performed. The amount of energy expended in a given task or occupation by different individuals has been measured in several instances but the results are rather conflicting. Much depends upon the previous training and experience of the individual, but even with individuals of similar history, the amount of energy expended in the same occupation varies tremendously (Becker and Hämäläinen, Lusk(*h*), 1917, Sherman(*c*), 1918, Benedict and Cathcart and Waller and associates(*a*)(*b*)(*c*)). To a considerable extent, this variation is probably due to differences in the amount of work accomplished, but other factors may also play a part. Nevertheless, it still remains true that typesetters and cobblers do less work than machinists and that business and professional men do not use their muscles as much as farmers or laborers. And, consequently, men whose occupations involve muscular exercise do not usually eat so much as do those who do much physical work. In some of the observations consolidated in Table IV, this fact may be obscured by three other factors. Of these, the influence of body weight has already been discussed. Of possibly equal significance is the fact that the reports are not only for individuals but for groups and families. The very large food consumption of a laborer doing hard work may no longer be so apparent when the only report is that for the food consumption of the family. It may be that the family of a man who is engaged in hard work will be similarly more active but it certainly is not always the case. (See also discussion of Sundstrom's results, pages 367-369.)

The influence of mental work upon food intake may be neglected. There is no evidence that mental work, even of the most fatiguing nature, appreciably affects the amount of metabolism. Starling(*b*) (1919) has suggested that mental work, while not requiring much energy, may require that to be supplied at a high pressure. This would justify a liberal protein and energy allowance in the food of brain workers.

Influence of Economic Status.—Last, but not least, is the economic factor. Beginning with Engel's figures and proceeding down the table, one can see that in every instance in which information as to income is included, except for one or two in the summary by Pearl, food consumption increases with increase in income. It is important to remember

this and to note that even in the neediest families studied, the energy content of the food does not fall below about 2500 calories per man per day, except in the case of those of low body weight, such as the Italians studied by Manfredi or the Japanese and Malays studied by others.

Amount of Protein.—The character of the food and, consequently, the relative importance of protein, fat and carbohydrate in making up the total energy content of the diet varies considerably with different peoples and different circumstances. But there are some quite evident uniformities and comparisons. Except in the most needy families, the protein content of the food anywhere in the world does not fall appreciably below one gram per kilo or 70 grams for the man of average weight in northern Europe and in the United States and it is generally as much as 1.3 to 1.5 grams per kilo, or 100 grams per man. The fraction of the total energy contributed by protein varies from 8.5 per cent in some Oriental diets and in those of some of the poorer classes in Europe to as much as 18 or 19 per cent in some of the Swedish and Finnish diets and even to 32 per cent in the case of the fishers at the mouth of the Volga who probably subsist largely upon fish and to 44 per cent among the Esquimaux (Krogh). But except for people under such unusual circumstances, the protein rarely contributes over 18 per cent and generally only from 12 to 15 per cent of the total energy. This comparatively narrow range is worthy of note.

Effect of Work.—Men at hard work eat more protein than do those not so engaged, but, apparently, this is due entirely to the greater consumption of food and not to a specific demand for protein or foods rich in protein. The fraction of the energy contributed by protein to the diet of men at hard work is frequently less than in the case of others of similar economic status and engaged at lighter work. This is most strikingly illustrated in the case of the diet of the Maine lumbermen in which the protein contributed only 10.5 per cent of the calories, a smaller proportion than was reported for any other group in the United States, except for some from the southern states. Similarly, the diet of lumbermen in the north of Sweden contained less than 10 per cent of the calories in the form of protein (only 8 per cent in the case of the man whose total was 9292, and 7.4 per cent for the two men whose average was 8439), whereas Hultgren and Landergren reported 16 per cent for Swedish working men and Sundstrom 15 to 16 per cent for the Finnish agricultural population.

Effect of Economic Status.—The *amount* of protein consumed is generally lowest with those of smallest income and grows larger with increasing income. But this increase is not indefinite and probably the total rarely goes above 160 grams or 2.3 grams per kilo. The relative importance of protein as a contributor of energy may, however, be slightly greater among the poor than among those of slightly greater income. A greater share of the necessary economy in food is attained at the expense of the fat. At the other end of the range of incomes, the proportion of energy

contributed by protein is apt to be slightly lowered by the increasing consumption of sugars and fats.

Amount of Fat.—The amount of fat consumed varies with the country, economic status, occupation and the time. Japanese diets seem to contain the least fat of any that have been studied, the maximum in the really native diets being about 30 grams, which is, or was, the recent European minimum. The fat consumption is also much lower in Italy, particularly among the laboring classes, than in northern Europe. Probably, this low fat consumption is, in both Italy and Japan, due to the general operation of the next factor to be considered, the economic.

In every series in which data are available beginning with Engel's of 1853, the amount of fat eaten increases regularly with the income. There are a few slight deviations from this rule in the series reported by Pearl and in the Scotch families of Lindsay but the number of observations included in these exceptional cases is rather small. In Pearl's series fat constitutes 37 per cent of the calories in the diets of the professional men. There is one group (salesmen) of higher income ($300 or 14 per cent more) in which fat contributed only 34 per cent of the calories but there are only five studies included in this group. In Lindsay's series, fat plays a slightly greater part in the diets of the families with income under 20s than it does in those of families with an income of from 20 to 25s, and as great a part as in the group with an income of from 27 to 31s, but the number of studies in these groups is only 5, 10 and 3, respectively.

The largest amount of fat is found in the diet of American and Swedish lumbermen, which, in one case, contained as much as 523 grams fat, furnishing 58 per cent of the calories. American athletes and Finnish students come next with 194 and 191 grams, furnishing 39 and 45 per cent of the total calories. In general, the amount and relative importance of fat in the diet increases with the total food intake, though in the diets of sedentary persons with ample income, the effect of the income may outweigh that of the energy intake as is illustrated in Ranke's and Neumann's observations on themselves (42 per cent and from 34 to 66 per cent, respectively).

During the fifty years immediately preceding the World War, there seems to have been a general increase in the amount of fat consumed, at least in several countries. Thus, Engel estimated the fat consumption in families whose income permitted saving to be 39 grams per man per day in 1853, but in 1899 found it to be not less than 56 grams in any group studied. The averages for all families were 28.5 grams in 1853 and 74.9 in 1891. In 1908, Slosse and van der Weyer found 74 grams to be the minimum in 33 studies of the diet of Belgian workingmen and Slosse and Waxweiler found that in only ten out of 1065 Belgian workingmen's families was it less than 35 grams and in only 132 was it less than 60 grams. Similarly, Lichtenfelt estimated the fat consumption in Germany in 1894

to be 94 grams per man per day, whereas Claassen, in 1907, estimated it as 141 grams (digestible) for the urban and 195 grams for the rural population. The Eltzbacher commission placed it at 139 grams for the population as a whole in 1912-1913. The series of reports from English cities confirm this tendency, though the number of observations is rather small. Thus in 1900, Paton, Dunlop and Inglis found that Edinburgh families with incomes of less than 20s used an average of 96 grams of fat per man per day; those with ample income used 92.3 grams. In Glasgow in 1911-1912, Lindsay found 76.3 grams in families with less than 20s income and 98 grams in those with an income of from 27 to 31s. In 1916, also in Glasgow, Ferguson found it at the same level, though the wartime restrictions on the use of fat might have been expected to reduce the figure. The high value, 88 grams, calculated by Dunluce and Greenwood from Rowntree's reports for York families with incomes of less than 26s weekly seems to be due to some local factor. It is greater than that reported for similar families in Edinburgh or Glasgow and much greater than that calculated by Dunluce and Greenwood from the Board of Trade returns for a large number of cities in Great Britain. It is interesting to note that the northern counties reported a higher fat consumption among the agricultural laborers than did the other counties of England. Within Rowntree's series, the usual economic effect is observed.

The amount of protein and of fat and their contribution to the total energy of the diet having been discussed, little remains to be said regarding the carbohydrate, save that it furnishes the remainder of the energy, from 400 to 600 grams per man per day being required. The increasing consumption of cane sugar is discussed on pages 395 and 397.

Ash Constituents.—Comparatively few studies of normal or customary diets have included determinations or calculations of the amount of the inorganic constituents. Tigerstedt(*e*) (1911) had the samples collected by Sundstrom(*b*) (1908) in his study of the diet of the Finnish agricultural population analyzed for some of the ash constituents with results shown in the first part of Table IV. The figures following were calculated to European body weights from Japanese diets by Rubner (*bb*) (1920). These are followed by those obtained by Nelson and Williams in a study of the calcium content of the urine and feces of four normal men (U. S.) on their accustomed diets. Then come the figures calculated by Sherman(*c*) (1918) for 150 supposedly typical American dietaries, and, finally, those calculated by Blatherwick for 32 studies in army training camps and by Howe (reported by Blatherwick) for four others. The enormous difference between the calcium and phosphorus contents of the Finnish and the American and the Japanese dietaries is due to the great difference in the amount of milk consumed. (See also page 415, for Rubner's calculation of inorganic food constituents in Germany before and during the war.)

Many investigators have observed and calculated the contributions made by animal and vegetable material to the total food. Particular importance has been attached to the content of animal protein, which has been regarded as far superior to vegetable protein. More recent investigation has indicated that this distinction is not altogether justified. It is true that animal proteins are, as a class, rather more effective as builders of body protein than are vegetable proteins but there are marked exceptions. Thus gelatin is the classic example of an incomplete protein whereas the protein of the potato is one of the most efficient (Hindhede(*c*) 1913, Rose and Cooper). Isolated plant proteins such as gliadine or zein may be very inadequate but the mixed proteins of wheat or of maize, as found in flour or meal, will maintain nitrogen equilibrium at a fairly low level, particularly if the whole grain be used or if it be supplemented by a small quantity of other proteins such as those in milk. In any *mixed* dietary, even if wholly of plant origin, the proteins are almost sure to be sufficiently varied to compensate for any individual inadequacies if only the total amount of protein be sufficient. Therefore, no attempt has been made to indicate in Table IV the quantity of animal protein consumed. However, in many cases, that can be calculated from the figures given for protein from meat and from milk and its products.

But the source of the protein, while of itself of not so great significance, is important as an indication of the amounts of those little known substances, variously denoted food accessories, food hormones, protective substances or vitamines, that may be present. Some idea of the inorganic content of the food may also be obtained in this manner. For this reason, there have been included in Table IV, where the data were available or could readily be calculated, the contributions made to total protein and, in some cases, to total energy also, by each of the classes of food materials, as was done in Table III. The same differences that were evident in Tables II and III also appear in Table IV. In addition, there are differences due to occupation, economic status, etc., most of which have already been discussed.

In all regions and at all times, man seems to have sought and found some beverage, other than water, to use with his meals. Meat, ale, milk, (sweet and fermented), wine, coffee, tea, cocoa and many others have been used. Particularly striking is the use of four plants of widely different botanical nature but all containing caffein or a related substance.

Changes in Food Habits within Recent Times.—The introduction of new foods as a result of the importation of new species, the improvement of old, or the development of transportation may greatly modify the food habits of a people. Maize and potatoes, unknown before the discovery of America, are to-day two of the staple crops of Europe and are fundamental to the economy of several countries. The improvement of the sugar beet has helped to lower the price of sugar and,

TABLE VI.—ASH CONSTITUENTS OF ORDINARY DIETS

FINNISH AGRICULTURAL POPULATION, WOMEN, 25 OBSERVATIONS ON 21 PERSONS, 7 DAYS EACH, ANALYZED. (*Sundstrom 1908*)

SUBSTANCE	PER WOMAN PER DAY			PER 3000 CALORIES		
	Minimum Grams	Maximum Grams	Average Grams	Minimum Grams	Maximum Grams	Average Grams
Calcium	1.13	3.86	2.28	1.50	4.17	2.79
Magnesium	0.21	1.14	0.66	0.50	1.11	0.84
Phosphorus	1.69	4.25	2.76	2.52	4.54	3.34
Rest	15.44	43.48	27.75			

FINNISH AGRICULTURAL POPULATION, MEN, 14 OBSERVATIONS ON 11 PERSONS, 7 DAYS EACH, ANALYZED. (*Sundstrom 1908*)

SUBSTANCE	PER MAN PER DAY			PER 3000 CALORIES		
	Minimum Grams	Maximum Grams	Average Grams	Minimum Grams	Maximum Grams	Average Grams
Calcium	1.92	9.85	3.79	1.68	5.13	3.06
Magnesium	0.73	1.39	1.09	0.69	1.02	0.85
Phosphorus	2.79	6.00	4.32	2.05	4.21	3.37
Rest	28.92	62.79	42.26			

JAPANESE DIETS, CALCULATED TO EUROPEAN BODY WEIGHTS BY RUBNER (1920)

SUBSTANCE	Minimum Grams	Maximum Grams	Average Grams
Calcium			0.281
Magnesium			0.414
Phosphorus			2.12
Potassium			2.81

FOUR AMERICAN MEN, SIX STUDIES OF FIVE DAYS EACH, ANALYZED. (*Nelson and Williams*)

SUBSTANCE	Minimum Grams	Maximum Grams	Average Grams
Calcium	0.676	1.016	0.852

150 AMERICAN DIETARIES, CALCULATED. (*Sherman, 1918-B*)

SUBSTANCE	PER MAN PER DAY			PER 3000 CALORIES		
	Minimum Grams	Maximum Grams	Average Grams	Minimum Grams	Maximum Grams	Average Grams
Calcium	0.24	1.87	0.73	0.35	1.47	0.73
Magnesium	0.14	0.67	0.34	0.17	0.53	0.34
Potassium	1.43	6.54	3.39	1.63	5.27	3.40
Sodium[1]	0.19	4.61	1.94	0.22	4.83	1.95
Phosphorus	0.60	2.79	1.58	0.72	2.30	1.59
Chlorin[1]	0.88	5.83	2.83	0.83	7.26	2.88
Sulfur	0.51	2.82	12.8	0.80	2.35	1.30
Iron	0.0080	0.0307	0.0173	0.0090	0.0234	0.0174

[1] Does not include salt added to food. Consequently is much too low.

TABLE VI.—ASH CONSTITUENTS OF ORDINARY DIETS

32 Army Organizations in Training Camps, Calculated. (*Blatherwick*)

Substance	Minimum	Maximum	Average
Calcium	0.374	1.060	0.711
Phosphorus	1.516	2.845	2.171
Iron (only 25)	0.0203	0.0494	0.0291

Four Infantry Companies of Same Regiment at Camp Cody during Same Period of 7 Days Calculated by Howe, Published by Blatherwick

Substance	Minimum Grams	Maximum Grams	Average Grams
Calcium	0.416	0.542	0.493
Phosphorus	1.662	1.801	1.731
Iron	0.0210	0.0221	0.0216

in that way, has helped make what was formerly a luxury, a relatively cheap and common food. The consumption of sugar within the last century increased tremendously throughout the western world, though some countries consumed more than others. The United States appears to lead the world in the per capita consumption of sugar, with Great Britain a close second. Whether or not this large consumption of sugar is desirable or not is still a moot question.

As one result of freeing populations from dependence upon local sources of supply, the development of transportation and refrigeration has helped to change the character of the food, particularly in making fresh foods available throughout the year and in giving the rest of the world access to the products of tropical and semi-tropical countries.

But these beneficial effects have been very largely confined to the cities and towns. In rural regions, the same causes seem to have led to less desirable changes. Instead of diversified farming, the tendency has been towards a "one crop" or "cash crop" agriculture. Under such a system the farmer no longer raises much of his own food but has only one crop which he sells for cash, with which he buys his food. He buys the purified, staple and stable foodstuffs and loses many valuable food constituents. The development of transportation and industry has not yet made it possible for him to buy, in addition to the staple foods, the fresh vegetables, etc., that he also needs. Sometimes, too, ignorant of the true values of foods, he may sell his own product to copy, through the village store, the habits of the city. To quote from Rubner(q) (1913): "I have noticed a very unfavorable influence of urban food requirements on the milk-producing districts of some regions of Switzerland, Germany, which is so characteristic that it deserves consideration. The milk-producing regions of the Bavarian highlands and of Switzerland had formerly an extremely, healthy, strong

and temperate population. Milk was largely used as a food, and the excess of production was placed on the market. In the course of years the communities gradually established central creameries in which the fat is withdrawn from the milk by means of centrifugal machines to produce cream and butter. The impoverished milk is partly returned to the farmers. The milk producers are paid in cash for their product, but a poor and insufficient food now takes the place of a former healthy one. The money now goes to the saloons. The potato conquers a new territory. Instead of the butter which was formerly used, cheap fats are now bought; in short, the change in diet is exactly such as we find with the poorer working population in the cities. The effects are exactly the same. Physical deterioration in such districts becomes more and more pronounced, reaching finally a low level. This is a very serious condition, which attracts attention and which must be combated by all possible means."

A similar effect seems to have been produced, in a rather different manner, in our own southern states. Formerly the corn was ground in small mills and all of it was used. Now much of it is sold for cash and "new process" or degerminated meal is purchased. It is quite possible that the present high freight rates will have one good result in encouraging diversified farming and the home production of more of the necessary food.

Indirectly, the improvement of transportation and the development of industry as a whole have helped to change food habits because of the improvement in economic condition. It is to this that we must ascribe the increased consumption of meat and fat in Germany and Belgium, and the gradual change from rye to wheat bread. The tendency to copy the diet of the wealthier classes is everywhere marked. The nature of this diet is determined largely by taste and fashion. The wealthier can, and do, secure for themselves the foods which have the more agreeable taste, and others, as soon as they can afford them, also wish to secure these foods for themselves. But taste will not alone explain the relative order of esteem in which foods are held. At one time shad, oysters and lobsters were so plentiful along the eastern coast of the United States as to be despised. To-day, they are delicacies. Diminishing supply may be responsible for this but not for all similar instances. Not all rare edible articles are highly esteemed foods. Nightingale tongues and peacock breast are no longer prized as they were in imperial Rome. Again, it is not so many years ago since calf thymus glands could be had at New York slaughter houses for the asking. To-day they are the expensive sweetbreads. That complex of varying influences that we call fashion has helped determine man's food habits as it has his other social practices. (See also Fairchild.)

Canned foods, while adding tremendously to the variety of foods available, have, to the extent that they have replaced fresh food, tended to re-

duce the narrow margin of intake over requirement of protective substances or vitamines.

A factor of considerable importance is the effect of advertising in accelerating and initiating changes in the character of the foods employed. The sales of specific articles of food can be as greatly stimulated as can those of any other commodity. Some of this advertising may be of quite a misleading character, even though the specific statements be absolutely true. Thus, butter substitutes are advertised as "purely vegetable" or as containing only vegetable fats, as if this were an advantage when it is exactly the opposite for vegetable fats do not contain an important substance which is present in most animal fats, particularly in butter.

Due to a combination of the factors already considered, grains are no longer ground at, or near, the place of consumption. The appearance and the keeping qualities of the product must be carefully considered. As a result, rice is polished and the germ is carefully removed from wheat and maize. But the diet that was adequate when more than half of it consisted of the entire grain may no longer serve to maintain the race in health and vigor if half the food consists of only part of the grain, for the two parts differ widely in composition. See Chapter on vitamins. (For further discussion of changes in food habits see Lichtenfelt(*c*), 1913, Rubner(*r*), 1913, Grotjahn, and Mendel.)

We have now considered the actual food consumption of man in different parts of the world as reported by many observers and have noted certain similarities, many differences and a number of progressive changes of quite general significance. To what extent are these resemblances to be considered as evidences of real physiological need? Is man's appetite a proper measure of his food requirement? Need we eat so much or should we eat more? Which is preferable, the high meat diet of the English speaking peoples and of those of the Argentine, the bread and milk diet of Finland or the comparatively meat- and milk-free diet of Japan?

Vegetarianism

First comes the question of vegetarianism. Space does not permit a full presentation of the benefits claimed to follow the exclusion of meat from the diet. There can, however, be little doubt that vegetarians have performed many feats requiring much muscular energy and have, in several races and other competitive sports, made a very striking showing. But there can also be little doubt that vegetarians, as a class, are not distinguished for good physique or for exceptional strength and endurance. Such showing as they have made seems to have been due largely to the rigorous training earnest advocates of the cult have imposed upon themselves. (Caspari, Albu, Hindhede(*a*)(*c*)(*d*), 1912, 1913, 1914.)

The argument that meat is not the "natural" food of man would seem to be fallacious. (Page 359.) Moreover, any such objection, if valid, would apply equally well to all cooked foods and, indeed, to all cultivated varieties of plants and throw us back upon the wild fruits of the forest and unbroken prairie.

The place of meat, as of any other food in the diet, is to be decided entirely upon physiological and economic considerations. Physiological investigations indicate no objection to the use of meat save in so far as the unduly large consumption of meat, in increasing the amount of protein, may be unwise. The economic objection is not so readily disposed of. The animals whose flesh is used for food return in that manner only a small proportion of the total energy they receive (Armsby). To a great extent, it is true, this is obtained from materials that are unfit for human consumption but to the extent that animals are fed grain, or other products of land that could be used to grow grain, vegetables or fruit, they do compete directly with man for readily utilizable foods. The loss in the animal in converting energy of the vegetable food into potential energy in the form of muscle and fat is one of the factors responsible for the comparatively high cost of meat in most countries. That is the objection to the free use of meats. So much of the income available for the purchase of food is spent for meat, which can be dispensed with, that not enough is left for milk and vegetables which are practically indispensable.

Benedict and Roth have shown that the basal metabolism of vegetarians is not appreciably less than that of meat-eaters. Unless the muscular systems of vegetarians are markedly more efficient than those of their fellows, the metabolism due to muscular work must be the same. Such economies in food consumption as are claimed for vegetarians and which the observations of Jaffa seem to corroborate must therefore be due to the operation of some other factor, probably the state of nutrition or level of metabolism. (Page 414.)

One of the great disadvantages of a vegetarian diet is its bulk. With the ordinary vegetarian diet, the work required of the digestive apparatus is considerably greater than with a mixed diet. This objection does not apply to the so-called lacto-vegetarianism, which permits the use of milk and eggs. Such a diet has much to commend it. It need not be bulky. The milk and eggs furnish protein of exceptionally good quality to compensate for possible deficiencies in those supplied by other articles of the diet. They contain much phosphorus and calcium, the latter of which is apt to be present in insufficient quantity if milk is not included in the diet, and furnish a considerable, if seasonably varying, quantity of some of the vitamines or protective substances. Moreover, the cow and hen return in the form of milk and eggs much more of the energy they receive than they do if kept for their meat (Armsby). In spite of what is often

said to be an uneconomical manner of distribution, milk is, for most people in this country, a comparatively cheap food.

Protein Minimum and Optimum

The question of the protein minimum and optimum has engaged the attention of physiologists for many years. While the necessity of a certain amount of protein has been recognized from the beginning, it has been believed that the optimum could be, and was, readily exceeded and that the excess was distinctly injurious. This belief has been due chiefly to the fact that protein is not completely oxidized to carbon dioxid and water, as are carbohydrates and fats, but leaves a non-combustible residue which must be excreted by the kidneys. Other objections are the high cost of protein foods, their ready susceptibility to putrefaction in the intestine and the fact that only a small part of the potential energy in protein is available for work, the remainder being excreted as urea, etc., or useful only as heat. Since, as a rule, the latter is produced in excess of requirements, this part of the protein energy may also be regarded as lost.

There have been many experiments on the so-called nitrogen minimum —the minimum amount of nitrogen in the food required to maintain an equilibrium with that of the excretions. Sherman(*f*) (1920) has collected the results of 109 experiments in 25 different investigations of this nature and has calculated the values found to a uniform basis of 70 kilos body weight. There is no difference in the per kilo requirements of men and women. The average of all 109 experiments is 44.4 grams. The range of values is very considerable, from 21 to 65 grams, but out of the 109 values, 94 fell between 29 and 56 grams, with an average of 42.8 grams, and 76, derived from 19 investigations and including 20 men and 4 women as subjects, fell between 30 and 50 grams, with an average of 40.6 grams. Expressed in terms per kilo body weight, these averages become 0.635, 0.61 and 0.58 respectively. Most of these experiments were of comparatively short duration and consequently the values obtained must be regarded as absolute minima and not as satisfactory and altogether sufficient amounts.

The apparently low protein intake of the Japanese and other Oriental peoples has long been noted but the earliest observations of any degree of accuracy seem to have been those of Eijkman on the diet of Japanese prisoners and those of Nagase on the diet of a military colonist in Formosa. (Both cited from Oshima.) In the latter, the content of protein was about one gram per kilo body weight. It was about the same in the diets of the prisoners doing no work but was higher (1.18 grams) in the diets of those doing light work and still higher (probably 1.5 grams or more) in the diets of those at hard work. These diets were not "freely chosen"

but were probably not greatly different from those to which the men had been accustomed.

In 1890 von Rechenberg published the results of his studies of the families of hand weavers in Zittau, a small town in Germany. The average intake of protein was 1.14 grams per kilo, but the condition of the people indicated that they were undernourished. They were very poor and their diet was not at all what they would have selected had they enjoyed better conditions.

Neumann's Experiments.—Neumann's studies on himself were really the first to show that so low a level of protein metabolism could be obtained on a mixed diet and maintained for a considerable period without evidence of ill effect. The diets were such as he had been accustomed to, although necessarily restricted in variety during the course of his studies, for he analyzed many of the foods himself. The first experiment included 305 consecutive days. In the following year there was a second experiment of 120 days. Three years later (four years after the first) a third study was begun. With the exception of November, December and January, this extended from May 1900 to June 1901. While reported as one experiment of 321 days, it really consisted of two separate studies of approximately half that length. The protein intake in the first and third studies was approximately one gram per kilo and, in spite of the rather low content of energy, Neumann gained slightly in weight. There was no evidence of any ill effect.

In the second experiment referred to, all the foods used were analyzed and the nitrogen of the urine and feces was also determined. Neumann found that he lost nitrogen and weight on the food as he then selected it and retained both only on a rather higher level of protein and energy intake than in the previous experiment. The values now obtained over a suitable period of 15 days were 1.16 grams protein and 40 calories per kilo per day. It seems probable that a consistent error was responsible for the much lower values for energy content in the other, not carefully analyzed, diets.

Chittenden's Experiments.—Very soon after the appearance of Neumann's paper, Chittenden published the results of his long-continued observations on himself, his friends and associates, on college athletes and on a group of soldiers. The experiment on himself was begun when he was 47 years old and weighed 65 kilos. He gradually reduced his diet until, eight months later, he weighed only 58 kilos. By that time an arthritis had disappeared, not to return, and he no longer suffered from headaches and bilious attacks which had formerly appeared periodically. He was able to do as much physical work as formerly with less than the customary degree of fatigue and muscular soreness. Observations during the following year showed that the nitrogen of the urine averaged 5.69 grams per day and that the intake with the food was approximately

one gram more or 6.69 grams per day. Similar experiments on his friends and associates gave similar results. The body weight fell slightly and then remained stationary. For long periods the nitrogen in the urine remained at a fairly constant low level, which was not so low, however, except with Mendel, as it was with Chittenden. The average for all, including Chittenden, was 0.117 gram nitrogen per kilo per day or the equivalent of 0.74 gram metabolized protein per kilo per day.

Experiments in which the nitrogen of the food, as well as that of the urine and feces, was determined gave similar results. The energy content of the food was not determined by analysis but was calculated from the results of published analyses. This involved a considerable degree of error, with such complex mixtures as were here employed.

In calculating the nitrogen balance, the nitrogen of the perspiration was not included. With men engaged in sedentary occupations, the amount of this was probably not great but it may very well have been large enough in May and June to have wiped out the apparent positive nitrogen balance (0.38 and 0.35 gm., respectively) in the second experiments with Mendel and Beers and to have increased the nitrogen loss in the corresponding experiment with Chittenden and Underhill. Moreover, the small gain of nitrogen, even if entirely real, is none too large when it is remembered that in other similar periods there was a greater loss. Taking all nine experiments together there was an average loss of 0.329 gram nitrogen per man per day, with an intake of 0.125 gram nitrogen and 32.0 calories per kilo per day. Practically the same values, 0.133 gram nitrogen and 32.4 calories, were obtained in the four experiments with positive nitrogen balance. For a man of 70 kilos, these values would become 58 grams protein and 2338 calories.

Eight athletes were under observation for five months and during the last two months of this period the average daily nitrogen excretion in the urine was 0.127 gram per kilo. Seven of these subjects were used in a seven day metabolism experiment. Considering all the results, there was an average daily loss of 0.06 gram nitrogen (not including that in the perspiration) per man upon an average daily intake of 0.147 gram nitrogen and 38.4 calories per kilo. Considering only the four experiments in which there was a positive nitrogen balance, the values were 0.158 gram nitrogen and 41.4 calories per kilo. For a man of 70 kilos, these would correspond to 69 grams protein and 2898 calories. It is interesting to note that the ratio of nitrogen : calories was lower in the food of the athletes than it was in that of the teachers. Notwithstanding the fact that these athletes had previously been accustomed to a high protein diet, they suffered no ill effect other than a slight loss in weight which may even have been advantageous and continued to increase their muscular strength, as measured by appropriate tests.

A detail of soldiers of the Medical Department of the United States

Army was sent to New Haven as subjects for Chittenden's experiments. The observations upon them differed from those upon the students and officers of the university in that the diet was prescribed. After about two weeks upon their accustomed rations, the food was selected by Chittenden to contain less protein and to furnish a rather smaller amount of energy, while retaining approximately the same bulk and furnishing considerable variety. Per kilo of body weight, the average daily urinary nitrogen, over a period averaging 144 days varied from 0.106 to 0.148 gram, the average of all being 0.128 per kilo or 7.89 grams per individual. The weight of the men remained nearly constant, some gained a little, others lost, but the losses were advantageous rather than otherwise. The men were regularly engaged in drill and other exercises and improved progressively in muscular strength and general physical condition during the whole of their stay in New Haven.

These observations were confirmed by three metabolism experiments, In the first, of six days' duration, each man's food contained a daily average of from 7.71 to 8.23 grams nitrogen, or from 0.111 to 0.153, averaging 0.135 gram, per kilo and furnished approximately 2078 calories, or 33 per kilo. In all cases the excretion of nitrogen in the urine and feces was greater than the intake in the food. Six weeks later, a second experiment of seven days was begun. The food now furnished 2509 calories or 40.4 per kilo and contained from 9.27 to 9.64 grams nitrogen, or from 0.128 to 0.180, average 0.157, grams per kilo. Upon this diet, all the men but one gained nitrogen, the average retention being 0.591 gram per man per day. A third experiment of five days came a month later. The energy content of the food was approximately 2840 calories or 45.9 per kilo and it contained from 8.14 to 8.67 grams nitrogen, or from 0.112 to 0.157, average 0.139, gram per kilo. Three men retained nitrogen and eight men lost, the average of all being a daily loss of 0.254 gram per man per day.

Since these losses occurred in spite of the fact that the diet furnished 300 calories per man, or 5 per kilo, more than that employed in the previous experiment, it would seem that the nitrogen of the food had been reduced to too low a level. The apparent nitrogen retention in the second experiment, 0.591 gram per man per day, is probably not much, if at all, greater than would be accounted for by the perspiration in men engaged in as much exercise as was taken by these subjects. We may therefore conclude that the least adequate nitrogen intake demonstrated by those experiments upon soldiers to be 9.5 grams, equivalent to about 60 grams of protein per day. Calculated to 70 kilos, it would be 69 grams. Similarly, the energy content would be 2800 calories. These values are very nearly the same as those obtained from the experiments upon athletes.

Although some of the food served was not eaten, the entire detail received practically the same diet. Nevertheless, as Benedict(*b*) (1906)

pointed out, there was a great variation in the amount of nitrogen in the feces of the different men, a variation which does not appear to have been observed in other experiments upon men receiving identical diets. During the first period the ratio of fecal nitrogen to food nitrogen varied from 9.06 to 24.6, average 18.0 per cent; in the second, from 10.7 to 24.4, average 17.6, per cent; and in the third, from 18.9 to 27, average 24.2, per cent. It varied in the same man in the different experiments. Benedict regarded these variations as evidences of a possible disturbance of the mechanism of absorption. But the variations in the case of the soldiers were greater than were observed with the professional men (from 10.9 to 19.0, average 15.1 per cent) or with the athletes (13.3 to 21.4, average 16.2 per cent) although the diets within these groups were not uniform. It seems to the writer that the irregular, and high, values for the nitrogen in the feces of the soldiers may have been due to the ingestion of additional food. That the men should sometimes have "broken diet" seems quite likely. If they did, they would have been likely to attempt to conceal their action by failing to collect all the urine or feces. At any rate, variations in the excretion of nitrogen in the urine such as were recorded in many instances and some of which are included in the following summary of the urinary nitrogen excretion on the last four days of the second balance experiment appear inexplicable except as a result of intentional, or accidental, failure to collect all the urine.

NITROGEN EXCRETED IN THE URINE OF

Nitrogen Intake	Oakman	Henderson	Morris	Coffman	Steltz	Lowenthal	Cohn	Zooman	Sliney	Broyles	Fritz
8.750	6.85	7.37 [1]	6.55	7.18	8.10 [2]	7.83	lost	7.56	7.78 [10]	6.18	7.05
10.427	7.95	8.22	*4.99*	7.93	*4.66* [3]	7.35	*5.59* [8]	7.51	7.49	7.68	lost
10.483	6.10	8.09	*5.38*	7.67	8.69 [4]	*4.29* [6]	9.55	7.08	7.54 [11]	*5.56*	8.71
10.265	7.96	8.20	7.01	7.95	8.20 [5]	8.07 [7]	6.77 [9]	6.81	8.23	7.69	*4.78*

Intake the same for each man, except as follows: 1. 8.555; 2. 9.30; 3. 11.107; 4. 10.024; 5. 10.392; 6. 10.654; 7. 10.886; 8. 10.215; 9. 8.164; 10. 8.164; 11. 10.475.

However, in spite of all the objections to some of the details, there can be no question but that Chittenden's results did show that it was possible for men to maintain themselves in good health and with a gain, rather than any demonstrable loss, in physical and mental vigor for a considerable period of time on diets containing less protein than had previously been considered necessary.

Fisher.—Chittenden's observations were extended by Fisher in his studies of the effect of diet upon endurance. It was found that students on a low protein diet, yielding only a moderate supply of energy, less than these students had been in the habit of obtaining, regularly increased in

their power of endurance in a number of physical tests. The experiments were not well controlled but they showed that healthy young men could live in an apparently perfectly healthy condition for at least two months on a diet containing only 0.97 gram protein per kilo per day.

McCay.—The advocacy of a low protein dietary was severely attacked by McCay, who based his criticisms chiefly upon the results of his experience in India. McCay found that Bengalis, as their incomes increased, partook to a larger and larger extent of protein food. The poorer classes, who were also in poorer health, subsisted chiefly on rice, with only small additions of meat, fish, milk or eggs. Some of his data and others calculated from them are included in Table IV. McCay emphasized the poor physical condition of those whose diets contained little protein as compared with that of those who, like the wealthier Bengalis, the Sikhs and others, ate more protein.

Perhaps the most striking of all McCay's studies is one upon Bengali and Anglo-Indian and Eurasian students at the same college. The former received a diet furnishing 3190 calories but only 67 grams protein, the latter, only 2822 calories but 95 grams protein. The average weight of the Bengali students was 54 kilos and they gained very little (less than one kilo) during their stay at the school, in spite of a gain of 1.5 to 2.5 inches in height. There was no increase in the girth of the chest. The Anglo-Indian and Eurasian students, however, gained an average of 8.2 kilos during the three years and their chest girths were increased by an average of one inch. While racial peculiarities may have had something to do with the result, it seems probable the difference in food played an important part.

However, since McCay's work was published, there has been an increasing recognition of the importance of, not only the amount of protein, but its kind, the nature of the constituent amino-acids, and of the significance of other dietary constituents. The diet of the Bengali (students and others) may well be criticized as containing not too little protein but possibly not enough of certain amino-acids, or even more likely, as being deficient in certain vitamines, or protective substances, or in one or more inorganic constituents.

Hindhede.—In a series of experiments designed to determine the minimum nitrogen intake required to maintain equilibrium, Hindhede(*c*)(*d*) (1913, 1914) succeeded in maintaining two men for considerable periods on diets containing rather less protein than those employed by Chittenden. The foods he used consisted of potatoes, or bread, with butter or margarin, with or without the addition of onions, plums, rhubarb or strawberries. The onions helped to make the large quantities of potatoes more palatable. The other additions acted as vehicles for sugar, thus permitting a reduction in the amount of bread. The nitrogen they contained did not appear in the urine but in the feces. Sometimes, indeed, the addition of plums,

rhubarb or strawberries to the food led to an increase in the fecal nitrogen greater than the total nitrogen of the added food. In this manner, these additions served to reduce the amount of what Hindhede regarded as "digestible protein," which he calculated from the difference between the nitrogen of the food and that of the feces. In this manner Hindhede was able to arrive at extraordinarily low figures for protein metabolism. But as pointed out on page 369, this procedure may not be justified and in the present discussion of Hindhede's results, the nitrogen of the food will be considered.

The lowest value for nitrogen intake, with maintenance of equilibrium, was obtained on the potato diet with 7.59 grams nitrogen or about 47 grams of protein for a man of 70.7 kilos. (The slightly lower value, 6.98 grams nitrogen or 44 grams protein, obtained in period E, was probably accompanied by a loss of nitrogen for the apparent gain of 0.2 gram nitrogen per day was scarcely sufficient to account for the loss in perspiration in the case of a man engaged in the hard work Fr. Madsen was then performing.) This appears to be the lowest protein intake, accompanied by a positive nitrogen balance, that has been recorded.

The analytical results reported by Hindhede cover a very considerable period, two years in the case of Fr. Madsen. It is difficult to extend quantitative observations over even so long a time as that and any of longer duration are almost impossible. But it should be remembered that Hindhede's subjects, particularly the two Madsens, were accustomed to a very low level of protein metabolism and were, nevertheless, healthy, vigorous men, well above the average in muscular development and endurance. Hindhede's own customary diet contained only 10.34 grams nitrogen or 64.6 grams protein per day and that of his family, which included children, only 75.7 grams per man.

The energy content of the food consumed by Hindhede's subjects appears to be rather high. It is possible that this low level of protein metabolism could be attained only at the cost of a large carbohydrate metabolism. However that may be, it is noteworthy that the very low protein metabolism observed in the case of the Madsens necessitated a very monotonous and limited dietary. Hindhede himself called attention to the difficulty of making a potato diet palatable or even endurable for any considerable period. It required the greatest care in the selection and preparation of the potatoes. On the bread diets, large quantities of sugar were required in order to maintain the energy yield of the food while keeping the protein content low.

As a matter of fact, unless unusual reliance be placed upon more or less purified foods such as starch, sugars and fats, it is nearly impossible to obtain 3000 calories without securing at the same time about 70 grams of protein or one gram per kilo. Reference to Table IV shows that this level is reached by all the dietaries reported, if only the energy content

is high enough. From Sherman's compilation (page 401) it is evident that this is 75 per cent or more above the minimum requirement. The danger of falling below the minimum protein requirement is, therefore, slight. As Bayliss said, "Take care of the calories and the protein will take care of itself." That is certainly true of the minimum for maintenance but it is not quite so evident that the optimum will be thus attained.

Liberal Protein Intake a Possible "Factor of Safety" (Meltzer).—In a memorable lecture delivered in 1906, Meltzer called attention to "The Factors of Safety in Animal Structure and Animal Economy" and suggested that the tendency of mankind to seek a level of protein metabolism above the minimum might be such a factor of safety. Just as we are provided with kidney, liver and lung tissue in excess of the apparent minimum requirement, so, too, the excess of protein above the minimum determined by experiment might serve as a factor of safety to cover emergencies and insufficiencies some of which we may not at present be able to recognize.

Aside from its value as a factor of safety, there are not wanting evidences of the desirability of a rather liberal supply of protein. Not only do the more vigorous and prosperous individuals consume a liberal allowance of protein but so also do the more vigorous nations. This may be effect rather than cause and, undoubtedly, is so in many cases with individuals. Meat and other protein foods are prized for a number of reasons including their agreeable taste, stimulating action, etc. This has led to a comparison of the desire for a liberal allowance of protein with the desire for alcohol. This seems to be based upon entirely too superficial resemblances. We now have a fairly good conception of how and why alcoholic beverages came to be so regularly employed by man. We know fairly well how they act to secure the effect desired. We know what are the consequences of excessive indulgence and even of the regular use of small quantities. We also know that not only scattered individuals for a few months or years but entire peoples for generations have maintained themselves in full health and vigor without the use of alcohol. There is today no such body of evidence in respect to the advantages of a low-protein diet. *Some protein is needed.* A slight, or even moderately great excess can scarcely be so very disadvantageous. When overindulgence in protein shall have been shown to be followed by ill effects at all comparable to those following the excessive use of alcohol, comparison will be in order but hardly until then.

Change of diet of whatever character has too often led to improvement in clinical condition for one to lay much stress upon the fact that Demuth observed such improvement on increasing the protein content of the diet of some of his patients. But such results as those reported by Moulinier with some 72 Indo-Chinese taken from Annam to the Yangtse valley as laborers are not so readily dismissed. The men first fed themselves as they had been

accustomed to at home, chiefly on rice. After several months, with the approach of cold weather, they tired easily and did very little work. They were then rationed and received 100 grams biscuit, 800 grams rice, 300 grams meat, 15 grams fat and 10 grams salt, yielding, in all, 3600 calories daily. Their capacity for work promptly increased and, when the meat ration was later diminished, the Annamese bought pork and poultry out of their own funds.

The following account of a similar instance is copied from Starling(*b*) (1919). "Thus Major Ewing relates how on a railway job in Canada, the Italian workmen were found to become inefficient at about 11 o'clock in the morning. These workmen were spending only seven to eight dollars for food at the canteen as against fifteen dollars expended by the Canadian workmen. The chief difference in the diet conditioned by this economy was in the meat. The company then insisted on the Italians spending fifteen dollars a month. With the extra money, they bought fat beef and it was then found that their work was entirely satisfactory." It may be objected that the favorable results in both these instances were due to the increased amount of food and not to the increased amount of protein. But, if the total amount of food had originally been insufficient, the men would, in all probability, have been hungry and would have eaten more.

Starling believes that the food of the Italians was originally too poor in fat and that the men felt the lack of this and responded to the addition of fat in the form of fat beef. But, while it is true that people accustomed to a liberal amount of fat suffer from lack of it, there is little reason to believe that its lack should inconvenience those, who like these Italians, probably never had any considerable amount of fat in their food.

A similar effect of meat feeding upon the laborers engaged in the construction of another railroad is mentioned by Collis and Greenwood (page 254).

Complete data are lacking but it seems to the writer that in all these cases the improvement was due to the increased protein content of the food. The original diets, while selected in accordance with previous habits, were possibly of not so high a protein content as in their native country. A change from unpolished rice to polished rice in the cases of the Annamese or from one kind of flour (as such or as bread or macaroni, etc.) to another with the Italians would have been quite sufficient to have produced an appreciable change in the protein content of the food.

It is curious that physiological literature should be so plentiful in arguments for a low protein diet based on the fact that protein is not completely oxidized but leaves a residue to be excreted by the kidneys. Why there should be so much solicitude for the kidneys rather than for other parts of the apparatus of metabolism is not entirely clear. Whatever may be the case in disease, it is yet to be demonstrated that the healthy kidney is in any way injured by being required to excrete 15, or even 20, rather

than 7 grams of nitrogen per day. A. and M. Krogh found no evidence of the prevalence of kidney disease, etc., among the Eskimos. There is rather more reason to be sparing in our use of the simpler carbohydrates, for it has now been demonstrated that a considerable number of individuals who would ordinarily be considered normal have rather a limited tolerance for sugars and that this tolerance can probably be impaired by continuously exceeding, or approaching, this limit. Apparently the factor of safety in the metabolism of glucose is less than it is for protein metabolism.

Fat Minimum.—During the war, and after, the importance of fat in the diet was greatly emphasized. The lack of fats was most severely felt by the people of central Europe and there were not a few who ascribed to their lack of fats the widespread occurrence of nutritional disorders, particularly "war edema." The Inter-Allied Food Commission adopted 2 oz (57 grams) of fat per man per day as the minimum upon which the peoples of the allied countries were to be asked to subsist. The absolute need of even so little is questionable. Experiments by Hindhede showed that his subjects could maintain themselves with much less fat. Fr. Madsen's diet included an average of 10.8 grams fat for 107 days. After a vacation of 21 days, during which he confined himself to a fat-poor diet, there was another period of 120 days during which the average fat content of the food was 13.9 grams. Then came another vacation of 21 days, then a period of 140 days with an average fat ration of 12.8 grams and then another vacation of 38 days. During both of these vacations, Madsen kept on a fat poor diet. Finally there was a period of 106 days with a diet containing an average of 14.2 grams fat. In all, he lived for over 18 months on a diet containing less than 15 grams of fat per day. Similarly, Holger Madsen ate food containing an average of 6.6 grams of fat per day for 117 days and, after a three weeks vacation, 7.9 grams fat for 180 days. After a two months vacation, there was another period of 106 days with an average of 7.5 grams of fat per day. The vacation diets were also poor in fat.

These results were not obtained in connection with the low protein diets previously discussed. Except for 30 days, Fr. Madsen's fat-poor diet regularly contained over 100 grams of protein and, during the period in which it fell below this level, Madsen lost weight. But whether this was due to the lack of protein and of fat or merely to the deficiency in energy content, which was at its lowest in this period, it is difficult to determine. Holger Madsen did not maintain his weight of 70 to 72 kilos on a fat-poor diet containing less than 90 grams of protein but, after his weight had fallen to 65 kilos, he maintained himself at this level and even gained a little on a diet containing 60 to 70 grams of protein, 6 to 7 grams of fat and furnishing 3000 calories.

Experiments by Osborne and Mendel on rats support these observations as do the observed dietary habits of Japanese and other Oriental peoples as well as those of the poorer classes in Europe. However, it seems probable

that, when the diet is deficient in fats, particularly in those of animal origin, it must contain considerable quantities of the green leafy vegetables as these and the animal fats appear to be the only sources of the fat-soluble vitamin or vitamins.

But if fat is not absolutely necessary, it is certainly very useful, for our whole accustomed cookery is dependent upon the use of fat. Without it, the housewife of western Europe and of the United States does not know how to prepare food nor does her husband relish it when it is prepared. Food prepared without fat leaves the stomach rapidly—it does not "stay with one." For those who require a large supply of energy, the use of fat is advantageous in that it supplies energy in a very concentrated form, nine calories per gram and all of it food, instead of four calories per gram, as with protein and carbohydrate, with each gram accompanied by from 0.5 to 9 grams water.

Carbohydrate Minimum.—Carbohydrates furnish more than fifty per cent of the energy content of most diets. If greatly reduced in amount, signs of defective fat metabolism may appear. However, the inhabitants of the arctic regions appear to maintain good health on diets containing very little carbohydrate. The possible ill effects of an excess of carbohydrate, particularly of the simple sugars, have already been mentioned (page 410) and are discussed more fully in the chapter on diabetes.

Minimum of Ash Constituents.—The requirements of the body for inorganic constituents have been, as yet, only scantily investigated and the demands for phosphorus and calcium have received the greater part of the attention that has been given to the subject.

Sherman(*d*)(*e*) (1920) has compiled the available data for these elements in a manner similar to that used in the determination of the protein requirement, to which reference has already been made. In 95 experiments included in 17 investigations (12 of which were by Sherman and his collaborators), the daily requirement of phosphorus varied from 0.52 to 1.20, with an average of 0.88 gram per 70 kilos body weight. Sherman states that "in a detailed study of the food supplies of 224 families or other groups of people selected as typical of the population of the United States only eight showed less than 0.88 gram of phosphorus per man per day and in all but two of these cases the phosphorus content would have reached this figure if the food consumed (without changing its character) had been increased in amount to a level of 3000 calories per man per day. The two cases which apparently contained less than the average actual requirement of phosphorus and would still have been thus deficient if the food had been sufficient in amount to cover the energy requirement amply were both reported from the southern states. . . . Outside of the southern regions where the food consists too largely of patent flour and new process (degerminated) cornmeal, the danger that a freely chosen American dietary will be deficient in either protein or phosphorus does not appear serious, in the light of our

present evidence, so far as the requirement of maintenance is concerned."

The compilation of the observations on calcium showed that in 97 experiments belonging to 14 investigations (10 of them by Sherman and his collaborators), the indicated daily requirement varied from 0.27 to 0.82, average 0.45, gram per 70 kilos. Sherman pointed out that, whereas only one out of 224 supposedly typical American dietaries fell below the indicated protein requirement, one in six was deficient in calcium. If all that fell below 3000 calories were increased to this level, none would be deficient in protein, but one in seven would still be deficient in calcium. It is interesting to observe, in this connection, that only one of Blatherwick's 32 army dietaries fell below 0.45 gram of calcium.

The possible occurrence of a calcium deficiency and consequent advisability of "liming the nation" seems recently to have attracted considerable attention in Germany. Rubner (1920) has considered the question and has concluded that with such foods as are used in Germany and are now available, there is no danger of a calcium deficiency for adults, so long as they get enough food to satisfy their energy requirements.

Rubner also calculated the values for the inorganic content of some Japanese diets to European body weights with the results shown in Table VI. The calcium content is much below Sherman's indicated requirement and is certainly considerably below that which was customarily consumed in Germany (page 415) but, if the analytical figures chosen by Rubner are correct, is certainly adequate *with Japanese dietaries.* It may not be with European food materials.

It is suggested by Rubner that the low fat content of Japanese diets may be related to their low calcium content. If they ate more fat (vegetable oils, etc.), the Japanese would not eat so much of their customary foods and would thus obtain even less calcium than they do now and might then suffer from a deficiency.

A certain absolute minimum of calcium and of other inorganic elements is unquestionably needed, but there are observations that indicate that this minimum may vary considerably under the influence of different factors. The first and most obvious of these is the texture of the food and the ease of digestion of the protein and carbohydrate contained therein. Hart, Steenbock and Hoppert found that cows and goats lost much less calcium on rations otherwise identical if they received fresh grass rather than hay. McCluggage and Mendel found that the calcium and magnesium of carrots and of spinach were poorly utilized by the dog. While it is true that Rose found that the calcium of carrots was as well utilized by women as that of milk, it, nevertheless, seems possible that in some other foods, less readily digested, some inorganic constituents are not made fully available for absorption.

The nature of the chemical combination in which the element appears

may play an important part. Organic iron is generally considered to be more valuable than inorganic, although the evidence is still conflicting. Also, although the requirement of the body for phosphorus may be met entirely by inorganic phosphate, it is possible that a larger amount is required than if some is present in organic combination.

Relation of Ash Constituents to One Another.—The existence of factors of quite different kind is indicated by the results of Bunge who found that the ingestion of potassium increased the excretion of sodium and by those of Hart and Steenbock who observed a similar effect, in swine, of the ingestion of magnesium upon the excretion of calcium. It is possible that some such action was responsible for the increase of 0.16 gram in the excretion of calcium in one of Sherman's experiments, following the addition of lean beef, containing 0.01 gram calcium, to the basal diet. The relation of the inorganic constituents of the food to one another is evidently of considerable importance.

Of all such relations, one of the most obvious, though not necessarily one of the greatest physiological significance, is the relation between acid- and base-forming elements. Sherman and Gettler first called attention to this. Blatherwick(*a*) (1914) showed that with some foods such as prunes and cranberries which contain considerable quantities of benzoic acid, which is not oxidized in the body but conjugated with glycine and excreted as hippuric acid, this may play a considerable rôle in the determination of the acid-base equilibrium of the body. Meats and cereals contain an excess of acid-forming elements, most fruits and vegetables an excess of alkaline, milk a slight excess of alkaline, and an ordinary mixed diet a slight excess of acid, elements. In his study of 32 army dietaries, Blatherwick(*b*) (1919) found a variation from an excess of acid equivalent to 39 c.c. normal acid to an excess of alkali equivalent to 2.4 c.c. normal alkali per man per day. The average of all was 2.2 c.c. normal acid.

Medical literature is rich in references to the supposed ill effects of an acid diet but most of these will not stand a careful examination. The fact that most organic acids are oxidized to carbon dioxid and water has generally been disregarded. Moreover, most of the evidence indicates that the body is able to neutralize the excess of acid that may be formed by neutralizing it with ammonia, at the expense of the urea of the urine.

Röse and Berg have reported that an acid-forming diet increases the need for protein. Their preliminary report is very interesting but acceptance of their views must await publication of their detailed results and confirmation thereof. Such confirmation would appear not to be forthcoming for Jansen (1919) and Fuhge have denied any such influence.

So little is known of the nature of the vitamins or protective substances that it is impossible to state with any degree of definiteness just what are the requirements for human nutrition. There seem to be at least three of these substances that must be supplied but there may be more. To what

amounts these are required we do not know. It is possible that these amounts will be found to vary considerably with the nature and amount of other constituents of the diet. Some evidence that this is so is already available. For a further discussion, the reader is referred to the chapter on vitamins.

Undernutrition

For years it has been known that fasting reduces basal metabolism but the significance of this fact as indicating a means of lowering the level of metabolism does not appear to have been fully appreciated until after the outbreak of the war. Then it was noted, particularly in Germany, that large numbers of people maintained themselves in good health and remained capable of performing their accustomed tasks while eating much less food than they had previously. They lost weight but not continuously and the loss was slight in comparison with the saving in food effected. The energy content of the food of the city population was probably about 2500 calories per man per day, but was increased by means of extra rations for those working in factories, mines, etc. (though still remaining below the accustomed quantity) and by extra foods purchased openly or surreptitiously by those whose means permitted them to do so.

Loewy and Zuntz showed that this maintenance at a lower level was due to lowered basal metabolism and not merely to the reduction in the protein of the food.

The success of the German people in maintaining health and vigor on such low diets appeared so striking that it seemed almost a foregone conclusion that their previous food intake had been greatly excessive.

In this country, Benedict, Miles, Roth and Smith, in a series of experiments, found that a group of twelve young men whose usual requirement of food was 3090 calories per day lost weight when placed upon a diet furnishing only from 1600 to 1800 calories, until after five weeks they had lost 10.5 per cent of their body weight. Thereafter, without changing the character of the food from that to which they were accustomed, they were furnished an average of 1967 calories, upon which the body weight remained stationary for a period of several months. Examination, by McCollum, of the diet furnished these men showed that it was not deficient in any known dietary constituent but only in total energy content. At first it seemed as if this economy in food was accomplished without any untoward effect but as the experiment continued it became evident that the men were not capable of the physical exertion that had previously been readily displayed. They lacked spirit and were easily tired. To use a colloquialism which many of them used to describe their condition, they lacked "pep." There was no clear evidence of lack of mental power but there was a very decided lessening of sexual desire.

Coincidentally, reports from Germany showed that similar effects, but greatly intensified, were appearing there. The early favorable results of a reduced dietary were found to be illusory and a very real failure to accomplish the usual amount of work was evident on all sides.

War Edema.—Outbreaks of what came to be known as "war edema" or "hunger edema" appeared in 1917 and became more and more frequent as time went on. The mortality figures soon showed an increase, particularly in the number of deaths from tuberculosis. A very good review of the supposed effects of the war diet on the incidence of disease was published by Determann.

Many factors have been held responsible for the appearance of "war edema." It is easy to point out some of these, such as the lack of protein and of fat (page 410), but there seem to be natural and experimental dietaries that share these deficiencies and that have been employed for long periods without producing edema. The large amount of water in the food has also been blamed. But Hawk and his collaborators found no such ill effect to follow the regular use of large volumes of water.

Rubner(*aa*) (1920) calculated the inorganic content of the rationed food of the German people in 1917-8 to be 3.375 grams K_2O, 0.226 gram CaO, 0.290 gram MgO, 0.089 gram Fe_2O_3 and 1.922 grams P_2O_5, per head per day. A similar calculation for the food used before war gave the following values: 4.403 grams K_2O, 1.221 grams CaO, 0.576 grams MgO, 0.154 gram Fe_2O_3 and 4.472 grams P_2O_5. The difference is marked. The calcium content of the war-time diet is far below Sherman's indicated requirement and is even less than that of Japanese diets, as calculated by Rubner.

When we consider how large a part the inorganic constituents of the body fluids play in determining their osmotic properties, it seems quite likely that a change in the inorganic content of the food, in which change the lack of calcium may or may not have been the significant factor, should have had some influence in the causation of the edema.

However that may be, lack of food—simple starvation—must be regarded as largely responsible, not only for war edema but also for the other disastrous effects observed. It is possible that a proper mixture of salts, vitamins and amino-acids added to the reduced diet would have prevented some of these, but for the present it seems safe to say that the only practicable way to secure these needed substances is to eat enough food of sufficient variety.

Probably the most complete and most accurate study of nutrition in Germany during the war, though limited to one individual, was made by Neumann upon himself. For seven months, from November, 1916, to May, 1917, he confined himself to the rationed articles with only such additions as were available to the poorer classes in his city (Bonn). This diet furnished him 45 grams protein, 18.9 grams fat, 287 grams carbohydrate

and 1546 calories daily. His weight fell from 167 to 127 pounds. (The chart is taken from Starling.) Other studies (Starling, Loewy and Brahm, Maylander, Mason) indicate that at about this time Neumann's diet was typical of that available to most of the city population. The well-to-do town dwellers and the agricultural population fared much better, the latter reducing their food consumption little, if at all.

The limitation of diet in the investigations of Benedict and in the experiences of the German people was accompanied by all the stimulation of war and the fervor of patriotic service. This may have helped to conceal from the subjects manifestations that might otherwise have been more promptly observed. In his studies of prison diets, Dunlop found that much smaller changes were promptly noticed by the subjects. He found that with a certain group on a diet containing 179 grams protein, 54 grams fat, 654 grams carbohydrate and furnishing 3928 calories, there was much waste and such complaints as there were regarded quality and not quantity. The ration was then reduced to one containing 165 grams protein, 56 grams fat, 566 grams carbohydrate and furnishing 3517 calories, which was tried for two months. By that time, 82 per cent of the prisoners of average weight (67 kilos) had lost weight. There was little waste but there were many complaints of lack of food. The ration was then increased to one containing 173 grams protein, 57 grams fat and 602 grams carbohydrate, furnishing 3707 calories. Complaints as to quantity ceased but there was no more waste than with 3517 calories.

There seems to be a certain definite level of nutrition to which the individual is accustomed and from which it does not vary over very considerable periods of time. Thus, Zuntz (Zuntz and Loewy(*b*)) found his basal metabolism the same after fifteen years. Any change in food intake from the amount required to maintain the level, assuming the amount of physical work performed to remain the same, is promptly indicated by a change in body weight which is, however, not continuous nor proportional to the change in the food.

It is interesting to examine in this connection the figures given in Table IV for two pairs of groups of dietary studies in the United States. The writer has selected from the studies of Atwater and Bryant in New York City in from 1896 to 1897 and from those of Wait in eastern Tennessee in from 1900 to 1904, those in which the weight and age of the children were given.[2] These were then separated into two groups, one of which included the studies of those families in which one or more children were at least ten per cent below the normal in weight as judged by Griffith's standards and the other in which all, or all but one in the case of large families, were of normal weight. The distribution of protein and calories is approximately the same within each pair. In New York, milk and its products sup-

[2] These are the only publications in which such information is given that are known to me.

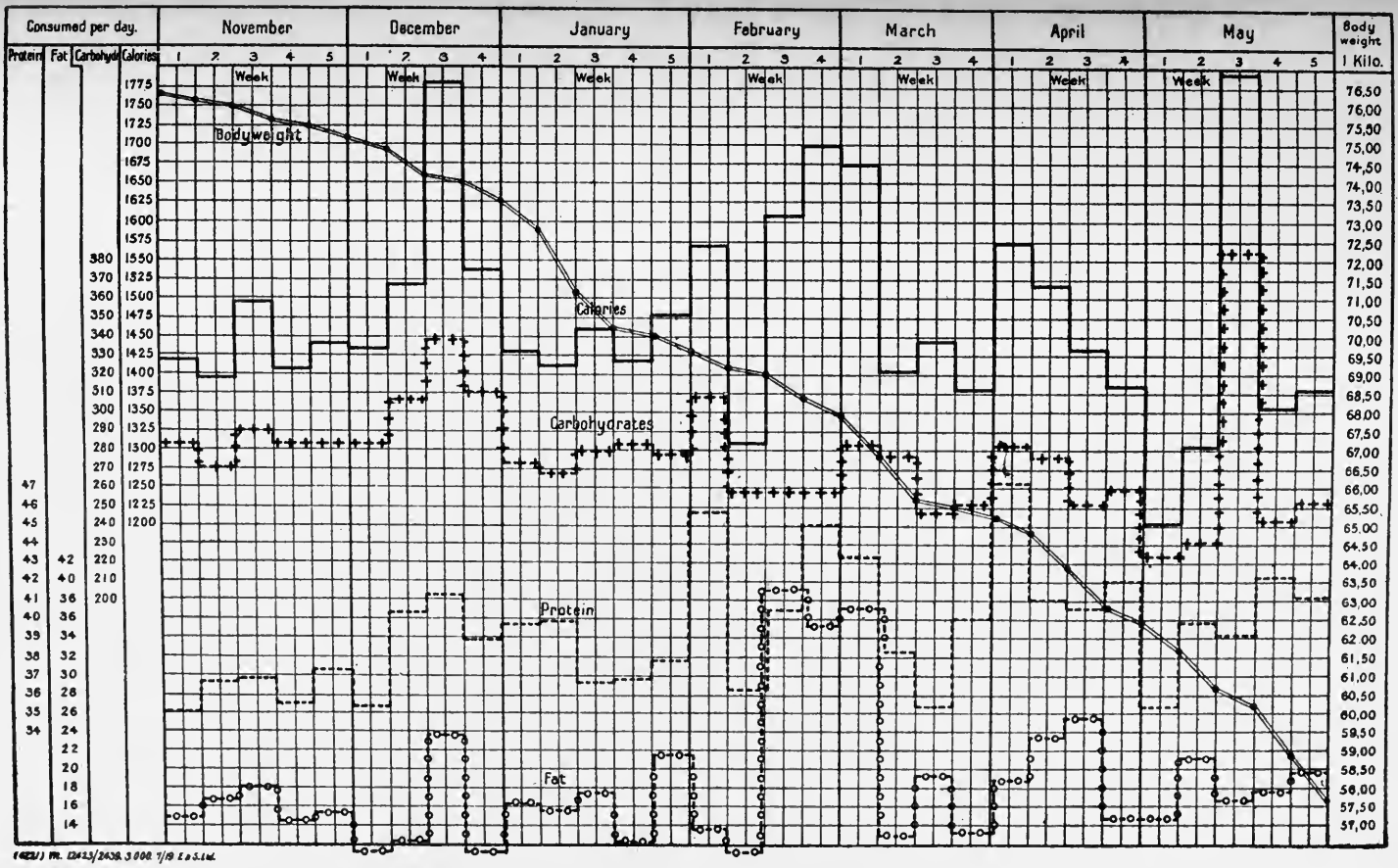

Chart III.—Neumann's observations on himself of reduced war diet. (Chart from Starling.)

plied less of the protein to those families whose children were below normal weight than it did to the other families, but these foods supplied more of the calories, indicating that the former group used less milk but more butter than the latter. The two Tennessee groups show no such difference in the consumption of milk and butter but, apparently, the families with children below weight used more peas and beans and less cornmeal than did the families whose children were of normal weight. But these differences are slight. The striking difference, in both pairs, is that in energy content, 8 per cent in Tennessee and 14 per cent in New York. *Food habits that do not secure to the ordinary family at least 3000 calories per man per day are, apparently, not suited to secure the proper development of the children.*

Of course, if no work is done, much less food is needed. This is indicated by many of the observations cited in Table IV and also by those of Benoit on a group of 78 Russian officers, prisoners in Germany, during a period of 480 days. Their food contained an average of 48.7 grams protein, 14.6 grams fat and 332 grams carbohydrate, furnishing 1697 calories per man per day. During this period, they lost an average of 140 grams. Although they had previously lost weight, they were still of about the "normal" weight, as judged from the American statistics, the average weight being 139 pounds (63 kilos) with an average height of 65 inches (1.65 meters). But they did no work and took very little exercise of any description. Bread and flour furnished 49 per cent of the protein, milk and its derivatives 23 per cent, meat and fish 16.3 per cent and vegetables 11.65 per cent. This was a very satisfactory distribution and no disturbances of nutrition were observed.

With the foods ordinarily consumed, the amount needed to maintain the body in its accustomed condition distends the stomach to a certain degree. If, with a change of diet, this bulk is lacking, the individual may be hungry, even though the energy content of the food is quite sufficient. On the other hand, in times of scarcity, the most varied, though indigestible and worthless materials are used simply to fill the stomach. Such is the case in Russia and in China to-day.

Bread and flour supply half the food of Europe. They are, ordinarily, the cheapest foods and in a time of high prices, their comparative importance increases and an adequate supply of bread becomes even more essential. Thus Miss Ferguson found that the same families in Glasgow used less meat, potatoes and sugar in 1917 than in 1916 but that they all used more bread and flour. It is not without reason that "bread" is so often used as synonymous with "food." A bread-eating people must have bread or suffer. For this reason, the most diligent attempts were made during the war to find suitable diluents or substitutes to use with wheat or rye flour in bread making.

A very complete study of the effect of a large number of such substances as were used in Russia in times of scarcity was made by Popoff.

An account of his experiments was published by Erismann in the *Zeitschrift für Biologie* in 1891. Notwithstanding this readily available account, many of these substances and many others were used in Germany during the war, some with very disagreeable consequences. Only two suitable substances appear to have been found. Blood obtained from slaughterhouses was, in this manner, made directly available as a food for man. Finely milled bran was also found useful. The addition of either of these made the bread less palatable than it formerly was. (Neumann(*d*) 1916.)

What is "Normal" Weight?—Such losses of weight as were observed in Germany and by Benedict and his associates in this country must be regarded as pathological but it is probable that if the reduction in the diet had not been quite so marked the loss in weight would have been much less. Benedict's subjects at an average weight of 66 kilos, were accustomed to a diet furnishing 3097 calories. A diet furnishing 1967 calories maintained them at about 59 kilos, indicating a loss in weight of 1 kilo for every reduction of 160 calories in the diet. If they had reduced the energy content of their food by 320 calories, or approximately 10 per cent, they would probably eventually have lost almost two kilos. If they had increased it by this amount, they would probably have gained about the same amount and would then have maintained themselves at this new level of metabolism and of weight. Which of these, 2777, 3097 or 3417 calories is the "normal"? That question cannot be answered until we know more definitely what is the "normal" weight for these men, 64, 66, or 68 kilos.

Symonds collected and published the height and weight of men and women at different ages as obtained from the records of accepted applicants for life insurance in the United States and Canada. The results are included in the following tables, the height including shoes and the weight, ordinary clothing.

TABLE VII.—SYMOND'S TABLE OF HEIGHT AND WEIGHT FOR MEN AT DIFFERENT AGES BASED ON 74,162 ACCEPTED APPLICANTS FOR LIFE INSURANCE

Ages	15–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69
5 ft. 0 in.	120	125	128	131	133	134	134	134	131	
5 ft. 1 in.	122	126	129	131	134	136	136	136	134	
2 in.	124	128	131	133	136	138	138	138	137	
3 in.	127	131	134	136	139	141	141	141	140	140
4 in.	131	135	138	140	143	144	145	145	144	143
5 in.	134	138	141	143	146	147	149	149	148	147
6 in.	138	142	145	147	150	151	153	153	153	151
7 in.	142	147	150	152	155	156	158	158	158	156
8 in.	146	151	154	157	160	161	163	163	163	162
9 in.	150	155	159	162	165	166	167	168	168	168
10 in.	154	159	164	167	170	171	172	173	174	174
11 in.	159	164	169	173	175	177	177	178	180	180
6 ft. 0 in.	165	170	175	179	180	183	182	183	185	185
6 ft. 1 in.	170	177	181	185	186	189	188	189	189	189
2 in.	176	184	188	192	194	196	194	194	192	192
3 in.	181	190	195	200	203	204	201	198		

TABLE VIII—SYMOND'S TABLE OF HEIGHT AND WEIGHT FOR WOMEN AT DIFFERENT AGES BASED ON 58,855 ACCEPTED APPLICANTS FOR LIFE INSURANCE

Ages	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64
4 ft. 11 in.	111	113	115	117	119	122	125	128	128	126
5 ft. 0 in.	113	114	117	119	122	125	128	130	131	129
1 in.	115	116	118	121	124	128	131	133	134	132
2 in.	117	118	120	123	127	132	134	137	137	136
3 in.	120	122	124	127	131	135	138	141	141	140
4 in.	123	125	127	130	134	138	142	145	145	144
5 in.	125	128	131	135	139	143	147	149	149	148
6 in.	128	132	135	137	143	146	151	153	153	152
7 in.	132	135	139	143	147	150	154	157	156	155
8 in.	136	140	143	147	151	155	158	161	161	160
9 in.	140	144	147	151	155	159	163	166	166	165
10 in.	144	147	151	155	159	163	167	170	170	169

From a study of the records of the relation of weight to height and of the mortality records, Symonds concluded that, below the age of about 30 years, those slightly above the average weight were the more likely to survive but that beyond this age those slightly under the average in weight showed the greatest vitality. But the optimum was very near the average. So that, apparently, the average weight of the people of this country is just about the "normal" in both senses of the word.

The relation of weight to height as calculated by Symonds is, of course, a rather crude measure of the state of nutrition or "degree of fatness" as Sherman calls it. Attempts have been made to devise others (Oppenheimer, Oeder) but these have not met with general acceptance.

Conclusion

From what has preceded, it is evident that it is impossible to fix definitely a "normal" diet. It is clear that its nature will depend upon geographical location, economic status, degree of muscular activity, habit, etc. Any diet that will maintain, or, rather, that has maintained normal health for generations must be considered to be a normal diet.

Judging by the experience of the race, checked by observations under laboratory conditions, or conditions approaching those of the laboratory, and by experiments upon animals, such a diet, if of European or American food materials, will furnish the man of 70 kilos engaged at moderate work 3000 calories and will contain from 75 to 120 grams of protein, at least 0.4 gram calcium and 0.8 gram phosphorus and will include a considerable amount of fruits and vegetables to furnish "roughage," vitamins, etc.

Success in maintaining individuals upon exceptional diets for even long-continued periods cannot be accepted as a criterion of the adequacy of a diet. Failure is proof that the diet is not satisfactory but success can only be taken to indicate exactly what was observed, which is merely that

no deficiency was detected within the period of observation. We now know that animals may be maintained in a satisfactory condition for periods corresponding to many years in the life of man upon diets that finally fail to continue to do so. Other diets will maintain the animal throughout life but will not permit reproduction. Experiments of comparable extent upon man are, of course, impossible. Custom, carefully observed and analyzed, must remain our chief reliance in deciding what is a normal diet.

As has already been shown, the cereals play a less important part in American diets than in those of most other peoples. It is probable that we shall, in the future, approximate them in this regard. Our per capita consumption of meat is almost certain to fall due to its almost inevitable increase in price, relative to other foods. What changes in our diet are physiologically sound and economically justifiable?

There seem to be two foods, or classes of foods, in which many American diets appear to be deficient or to approach deficiency. These are milk and its products and fresh vegetables, particularly the green leafy vegetables. Students of nutrition appear to be united in this opinion. Thus McCollum(*c*) wrote: "Milk is our greatest protective food, and its use must be increased." "There is no substitute for milk and its use should be distinctly increased instead of diminished, regardless of cost." "Milk is just as necessary in the diet of the adult as in that of the growing child." According to Lusk(*h*) (1917), the mother of a family consisting of two adults and three children should buy no meat until she has first bought 3 quarts of milk a day. Sherman(*c*) (1918) writes: "It therefore seems advisable to spend at least as much for fruit and vegetables as for meat and fish; also to spend at least as much for milk as for meat or for milk and cheese as for meat and fish." . . . "General adoption of a dietary such as we now believe to be best would call for more milk and perhaps more vegetables and fruit than now come to our city markets."

To quote again from McCollum: "In the light of facts presented in the previous chapters of this book, there can be no reasonable doubt that the importance of poor hygienic conditions and of poor ventilation have been greatly over-estimated, and that of poor diet not at all adequately appreciated as factors in promoting the spread of this disease." (Tuberculosis.)

It is probable that the importance of a faulty diet in reducing resistance to other infectious diseases has similarly been overlooked. Moreover, when we consider how slowly the signs of such unquestionably nutritional disorders as scurvy or rickets usually develop, it is not difficult to understand that a slighter nutritional deficiency may give rise to general inefficiency and impaired health.

We cannot hope to maintain and improve our standards of health and efficiency without maintaining and improving the character of our diet.

SECTION III

Body Tissues and Fluids.....*Victor C. Myers*

Composition and Significance of Blood—Blood Volume—Total Solids—Specific Gravity—Reaction and Hydrogen Ion Concentration—Blood Proteins—Serum Proteins—Fibrinogen—Hemoglobin—Blood Cells—Blood Nitrogen—Total Nitrogen—Non-protein Nitrogen—Urea—Uric Acid—Creatinin—Creatin—Amino Acids—Ammonia—Rest Nitrogen—Blood Sugar—Blood Lipoids—Total Fat—Lecithin — Cholesterol — Acetone Bodies—Mineral Constituents—Sodium—Potassium—Calcium—Magnesium—Iron—Chlorids—Phosphates—Sulphates—Blood Gases—Oxygen—Carbon Dioxid—Muscle—Liver and the Bile—Connective Tissues—Brain—Phosphatids—Cephalin—Cerebrosids—Sulphatids—Cholesterol—Extractives—Cerebrospinal Fluid—Saliva—Milk.

Body Tissues and Fluids

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So much attention has recently been devoted to the study of the chemistry of the blood that a consideration of the subject of the body tissues and fluids can hardly be made without undue emphasis on the blood. Some of the more recent methods have been applied to advantage in the study of spinal fluid and milk, and an extended application of many of these methods to the study of fresh autopsy tissues, muscle, liver, etc., would probably yield equally valuable results.

Composition and Significance of Blood

During the past decade, 1910-20, the chemical composition of the blood has been a topic of increasing interest and importance, quite eclipsing in significance the studies carried out on the urine during the preceding decade. In the case of urine the advances were primarily the result of the impetus furnished by the new methods of Folin and of S. R. Benedict, and these same workers, together with Van Slyke, are responsible for many of our new methods of blood analysis. During this latter period the blood has probably been the topic of more studies than any other body tissue, fluid or secretion. The practical importance now attached to the chemical examination of the blood would appear to be rapidly overshadowing the importance formerly attached to the examination of the urine.

Blood has often been referred to as a fluid tissue. That the blood may readily be compared with other tissues from the standpoint of its solid content is evident by the fact that in perfect health the total solids are only slightly less than those of the muscle tissue and even more than those of some of the glandular tissues of the body. According to recent observations human blood normally constitutes about 8.5 per cent of the body weight. Blood is the common carrier of nutritive materials to the various tissues of the body and waste products such as carbon dioxid, urea, etc., to organs of excretion. From this it is apparent that an inability to properly metabolize certain food materials or properly excrete certain waste products should result in changes in the composition of the blood. Owing to the various factors of safety in the body it would seem unlikely

COMPOSITION OF HUMAN BLOOD

Constituent or Determination	Calculated as	Normal		Pathological
		Range	Average	Range
Blood Volume { Plasma	Per Cent of Body Weight	4.5– 5.7	5.1	3.8 – 6.2
Blood Volume { Whole Blood		7.6– 9.1	8.5	4.3 – 13.7
Total Solids	Per Cent	19 –23	22	10 – 25
Total Serum Protein	" "	6.7– 8.2	7.5	4.2 – 9.1
Serum Albumin	" "	4.8– 6.7	5.6	3.7 – 7.0
Serum Globulin	" "	1.4– 2.3	1.9	1.7 – 2.6
Fibrinogen (plasma)	" "	0.3– 0.6	0.5	0.1 – 0.9
Hemoglobin (whole blood)	" "	12.5–23.0	16	3.5 – 24.0
Cells { Erythrocytes	Per cu. mm.	4,500,000– 5,500,000	.....	100,000– 12,000,000
Cells { Leucocytes	" " "	3,000–10,000	.....	500–600,000
Cells { Platelets	" " "	200,000–500,000		
Total Nitrogen	Per Cent	3.0–3.7	3.3	1 – 4
Total Non-protein Nitrogen	Mg. to 100 c.c.	25 –35	30	20 –400
Urea Nitrogen	" " " "	12 –15	15	5 –350
Uric Acid	" " " "	2 – 3	2.5	0.5 – 25
Creatinin	" " " "	0.5– 2	1.0	0.5 – 35
Creatin	" " " "	3 – 7	5	2 – 35
Amino-Acid Nitrogen	" " " "	4 – 8	5	4 – 30
Ammonia	" " " "	–0.1		
Rest Nitrogen	" " " "	4 –18	11	
Sugar (glucose)	Per Cent	0.09– 0.12	0.10	0.05– 1.30
Diastatic Activity	" "	14 –18	15	10 – 75
Lipoids				
Total Fatty Acids (whole blood)	" "	0.29– 0.42	0.36	to 6.10
Total Fatty Acids (plasma)	" "	0.30– 0.47	0.39	to 8.13
Total Fatty Acids (corpuscles)	" "	0.27– 0.45	0.32	
Lecithin { whole blood	" "	0.28– 0.33	0.30	0.16–0.46
Lecithin { plasma	" "	0.17– 0.26	0.21	0.14–0.50
Lecithin { corpuscles	" "	0.35– 0.48	0.42	0.34–0.70
Cholesterol { whole blood	" "	0.14– 0.17	0.15	0.06–1.00
Cholesterol { plasma	" "	0.15– 0.18	0.16	0.06–1.20
Cholesterol { corpuscles	" "	0.13– 0.17	0.14	0.10–0.20
Acetone Bodies	T'l as Acetone			
Acetone	Mg. to 100 c.c.	1.3 – 2.6	2	2–350
Aceto-acetic Acid	" " " "	0.3 – 2.0	1	2– 50
β-hydroxybutyric Acid	" " " "	0.5 – 3.0	1	1–300
Mineral Constituents		280–320	300	
Sodium (serum) as Na	Mg. to 100 c.c.	16– 22	20	10– 35
Potassium (serum) as K	" " " "	150–250	200	50–400
Potassium (whole blood)	" " " "	9– 11	10	2– 25
Calcium (serum) as Ca	" " " "	2– 3	2.5	
Magnesium (serum) as Mg	" " " "	.....	50	
Iron (whole blood) as Fe	" " " "			
Chlorids (whole blood) as NaCl	" " " "	450–500	470	350–700
	" " " "	570–620	600	500–850
Chlorids (plasma) as NaCl				
Phosphates as P				
Inorganic (plasma)	" " " "	1.5– 4.5	3	1– 40
Lipoid (plasma)	" " " "	5 –12	7.5	
Organic (corpuscles)	" " " "	40 –75	53	
Sulphates (whole blood)	" " " "	0.5– 1.0	0.7	0.5–16

Composition of Human Blood (*Continued*)

Constituent or Determination	Calculated as	Normal		Pathological
		Range	Average	Range
Blood Gases	Volumes Per Cent			
Oxygen				
Capacity	" "	19–23	21	7–33
Arterial Content	" "	18–22	20	6–32
Venous Content	" "	13–17	15	3–27
Carbondioxid				
Arterial Content (whole blood)	" "	45–55	50	
Venous Content (whole blood)	" "	50–65	58	
Capacity (plasma)	.....	55–75	65	5–90

that these changes should be very marked except in severe pathological conditions. With sufficiently delicate methods, however, these slight changes should be readily detected. The development of simple and very delicate colorimetric methods has done much to aid in this type of work.

More and more we have come to consider the various changes which take place in the body from a quantitative point of view. The various blood constituents, and certain blood determinations, with the range of their normal and pathological variations, are given in the table above.

Blood Volume.—Owing principally to the recent work of Keith, Rowntree and Geraghty the subject of blood volume has received considerable attention. These investigators have introduced a new method of determining blood volume and have obtained somewhat higher figures than those formerly given for man. The principle underlying their method is the introduction directly into the circulation of a non-toxic, slowly absorbable dye (vital red) which remains in the plasma long enough for thorough mixing, and the determination of its concentration in the plasma colorimetrically by comparison with a suitable standard mixture of dye and serum. According to this method the plasma normally constitutes approximately 5 per cent, or one-twentieth of the body weight. The volume occupied by the corpuscles was calculated with the aid of the hematocrite and found to average 43 per cent for the erythrocytes and 57 per cent for the plasma. On this basis Keith, Rowntree and Geraghty have calculated that blood normally constitutes 8.8 per cent or 1/11.4 of the body weight. With this method they were able to demonstrate the amount of decrease in blood volume as the result of hemorrhage and of the increase following intravenous infusion of saline.

Significant observations were made in a few pathological conditions. Both the blood and plasma volume are increased in pregnancy, before term, but return to normal within a week or two after delivery. In obesity the plasma and blood volumes are relatively small. Many cases of anemia

exhibit a relatively high blood volume, while in some cases polycythemia in the sense of a high blood count may be dependent on a low plasma volume. In anasarca accompanying myocardial insufficiency the blood volume may be absolutely increased. In many cases of marked hypertension the volume is small, indicating that hypertension is not necessarily dependent upon a large blood volume.

More recently a very elaborate study of the question of blood volume has been carried out on animals by Whipple and some of his coworkers. Since "vital red" was not available, their earlier experiments were made with "brilliant vital red." Later they tried out a very large series of dyes for use in this connection, and discovered a blue azo dye which appears to be slightly superior to the vital red group, especially as regards ease and accuracy of colorimetric readings. In the same series of papers McQuarrie and Davis have employed a method of determining blood volume which consists essentially in reading refractometrically the serum non-protein increase after the intravenous injection of a known amount of acacia or gelatin solution, or a mixture of the two. The results obtained were quite comparable to the dye methods and an acacia method described by Meek and Gasser.

The most recent publication on blood volume is that of Bock who presents some very interesting data, obtained with the vital red method, on five normal and twenty pathological cases. The constancy of the plasma volume under widely varying conditions is pointed to as a striking fact. Although the plasma volume remains practically normal in polycythemia and anemia, as shown by the table below taken from Bock, the total blood volume is increased in the former and decreased in the latter owing to variations in the cell content.

Data on Blood Volume

Condition	Number of Cases	Total Plasma Per Cent of Body Weight	Total Blood Per Cent of Body Weight	Hemoglobin Calculated from O_2 Capacity Per Cent	Red Blood Cells in Millions
Normal	5	5.1	8.2	119	4.8
Polycythemia	3	5.1	13.7	163	9.1
Pernicious Anemia ..	7	4.9	5.7	47	1.6
Miscellaneous	7	4.9	7.1	79	3.9
Diabetes	8	4.8	7.3	118	4.6

Blood volume methods have been critically discussed by Lamson and Nagayama, but the authors concede that the plasma volume method of Keith, Rowntree and Geraghty is as correct as any and the best method at our disposal for most purposes.

Total Solids.—Where a careful quantitative examination of the blood is being carried out, the estimation of the total solids is often of consider-

able value. In the first place, the solid content of the blood is a very excellent index of the functional condition of the blood, blood proteins and blood cells taken together, and furthermore is of value in explaining small fluctuations in the content of the individual constituents. Normally the total solids amount to from 19 to 23 per cent, although in primary and secondary anemia, severe nephritis, etc., the amount may be decreased to nearly one-half these figures. That the total solids may be increased in cholera, as a result of the severe diarrhea, was recognized by Carl Schmidt many years ago. An increase in the blood solids was found by Underhill to result from poisoning by the lethal war gases.

Specific Gravity.—The specific gravity of human blood in the adult male varies between 1.041 and 1.067, the average being about 1.055. For the female the figures are slightly lower. The specific gravity obviously varies in much the same way as the solids. The determination appears to be little used at the present time. Gettler and Baker have recently given some new observations on serum. They found the specific gravity of the serum of both men and women to range from 1.026 to 1.030, the majority being between 1.027 and 1.029.

Reaction and Hydrogen Ion Concentration.—Normal human blood as it exists in the body is faintly alkaline in reaction, i. e., it has a hydrogen ion concentration only slightly less than pure water, and this degree of alkalinity tends to be very constantly maintained under a variety of conditions. The blood itself, owing chiefly to the "buffer" action of the carbonates of the plasma and phosphates of the corpuscles, can take up considerable amounts of acid or alkali without much change in its reaction. An appreciable change in its hydrogen ion concentration indicates a failure of this protective mechanism and the presence of a severe acidosis. From a practical point of view the CO_2 combining power of the blood is much more useful, since the change occurs much earlier (see below).

As the result of a series of analyses on thirty normal individuals by the gas chain method, as described by Michaelis, Gettler and Baker found pH to range from 7.52 to 7.60 at 22°C. Levy, Rowntree and Marriott have described a very simple indicator method of determining the hydrogen ion concentration and serum. With this method oxalated blood from normal individuals gave a dialysate with a pH varying from 7.4 to 7.6, while that of the serum ranged from 7.6 to 7.8. In a small series of clinical acidoses, the serums varied from 7.55 to 7.2 and the oxalated blood from 7.3 to 7.1.

Blood Proteins.—Considerable experimental evidence has recently been adduced by Kerr, Hurwitz and Whipple (*c*) which points to the liver as being concerned in the maintenance of a normal level of the blood serum proteins (albumin and globulin). The evidence is not so convincing nor so striking as that obtained by Whipple for the plasma protein fibrinogen which has such an intimate relation to liver injury. In the case of the

blood serum proteins the stability of the normal level appears to be fairly well maintained under widely varying conditions of health and disease.

Serum Proteins (*Albumin and Globulin*).—The subject of the serum proteins in man has recently been very carefully considered by Rowe (*b*), who has employed the microrefractometric method of Robertson for their study in normal and a number of different pathological conditions. In a series of twenty-two normal cases the serum albumin was found to vary between 4.6 and 6.7 per cent, the serum globulin between 1.2 and 2.3 per cent, the total serum protein between 6.5 and 8.2 per cent and the nonproteins between 1.1 and 1.3 per cent, while the percentage of globulin in the total protein varied from 16 to 32 per cent. Muscular activity, even of the simplest sort, increases total serum proteins, this increase occurring more in the albumin than the globulin fraction. In three cases with severe muscular work Rowe (*c*) found the total protein increased from 1.1 to 1.9 per cent and the albumin from 0.8 to 1.5 per cent, while in one case with light exercise the total protein was increased 0.5 per cent and the albumin 0.3 per cent.

The following table compiled from data given by Rowe gives a comparative idea of the blood serum proteins in the normal human subject and in a variety of pathological conditions.

Blood Serum Proteins in Health and Disease (Averages)

Condition	Number of Cases	Albumin Per Cent	Globulin Per Cent	Total Protein Per Cent	Globulin to Total Protein Per Cent
Normal subjects	22	5.6	1.9	7.5	22.5
Syphilis	19	5	2.5	7.5	34
Pneumonia	8	3.7	2.5	6.2	40
Chronic nephritis with edema	3	2.5	1.7	4.2	40
Chronic nephritis with uremia	5	4.2	2.3	6.5	35
Chronic nephritis without uremia or edema	7	4.5	2.2	6.7	33
Cardiac decompensation	9	4.7	2.6	7.3	36
Arteriosclerosis	9	4.8	2.3	7.1	32
Diabetes	10	5.5	1.9	7.4	26
Anemia	9	3.9	1.7	5.6	30

From the above it is apparent that in syphilis the globulin is definitely increased, while the total protein remains about normal. In pneumonia the globulin is increased more in relation to the total protein than in syphilis, while the total protein is reduced, due probably in large measure to a dilution of the blood serum by water retention, which occurs in fever. The lowest values for total serum proteins are obtained in chronic nephritis with edema, due probably to chronic intoxication as well as hydremia.

In chronic nephritis with uremia the total proteins may be nearly normal but the globulin is usually increased. Except in very severe diabetes the findings are practically normal. In pernicious anemia the total proteins are not as low as would be expected from examination of the whole blood, being higher than in nephritis with edema.

Fibrinogen.—According to Whipple the normal fibrinogen limits for the human subject may be given as 0.3 to 0.6 per cent with an average of 0.5 per cent per 100 c.c. of plasma. In pneumonia and septicemia fibrinogen is much above normal, reaching 0.9 per cent, while in acute liver injury it drops to a very low level or even zero in some fatal cases. In chronic liver disease fibrinogen often falls markedly and may cause bleeding (cirrhosis). In general cachexias, such as sarcomatosis, nephritis and miliary tuberculosis, the fibrinogen may be quite low, 0.1 per cent.

Hemoglobin.—Hemoglobin is the iron containing and oxygen carrying pigment of the red blood cells. It is a conjugated protein, composed of the histon, *globin, and hemochromogen,* the iron containing pigment. In the presence of oxygen the latter body is rapidly transformed into *hematin.* Hemoglobin is crystallizable, and peculiar in its high iron content, which amounts to 0.34 per cent. Under normal conditions it is quite completely saturated (95 per cent) with oxygen in arterial blood, although in the case of venous blood the oxygen is ordinarily reduced to about 75 per cent of saturation. Owing to this fact the hemoglobin of the blood may be more correctly referred to as oxyhemoglobin. Oxyhemoglobin has a bright red color but (reduced) hemoglobin is darker and more violet or purplish, hence the darker color of venous blood. For further properties of hemoglobin and its many derivatives reference may be made to Hammarsten.

The estimation of hemoglobin was apparently the first chemical determination in the blood to find extensive clinical application. It seems unfortunate that most of the estimations recorded should have been made employing an empirical scale with 100 as the normal, especially since the 100 is somewhat of a variable factor with different methods owing to different standardizations. The hemoglobin content of the blood varies widely not only in disease, but also in different age periods as recently pointed out by Williamson. For this reason it would appear more logical to record the hemoglobin, as we do other blood determinations, in grams per 100 c.c. or per cent.

The table below compiled from observations of Williamson well illustrates the changes in the hemoglobin content of the blood over different age periods. The figures were obtained with the accurate spectrophotometric method, fifteen or more of both males and females being employed for each age group. From this table it will be noted that during the first few days of life the hemoglobin content exceeds 20 per cent, but then drops rather abruptly the third month to below 14 per cent and does not pass this figure until the tenth year. During the adult period of life in

HEMOGLOBIN IN NORMAL MALES AND FEMALES DURING DIFFERENT AGE PERIODS

	Hemoglobin Gm. per 100 c.c. of Blood		
	Male	Female	Both Sexes
1 day	23.3	23.2	23.3
2 to 3 days	22.5	23.1	22.8
4 to 8 days	22.1	22.1	22.1
9 to 13 days	21.4	21.3	21.4
2 weeks to 2 months	18.7	18.0	18.4
3 to 5 months	13.1	14.3	13.7
6 to 11 months	13.2	14.2	13.7
1 year	12.8	12.2	12.5
2 years	12.4	12.7	12.6
3 years	13.2	13.1	13.2
4 years	13.3	14.0	13.6
5 years	13.8	13.3	13.5
6 to 10 years	14.6	13.7	14.2
11 to 15 years	14.5	14.9	14.7
16 to 20 years	16.8	15.6	16.3
21 to 25 years	17.2	15.0	16.0
26 to 30 years	16.4	15.5	15.9
31 to 35 years	16.9	15.4	16.2
36 to 40 years	17.0	15.4	16.2
41 to 45 years	16.9	15.6	16.2
46 to 50 years	17.1	15.5	16.3
51 to 55 years	17.0	16.1	16.6
56 to 60 years	17.0	15.8	16.4
61 to 65 years	16.5	15.7	16.1
66 to 70 years	16.2	15.5	15.8
71 to 75 years	15.2	15.5	15.3
76 and over	15.7	15.0	15.4

both sexes (from 16 to 70 years) the hemoglobin maintains a fairly constant level of about 16 per cent. From the third month to the fifteenth year the values obtained in the female appear to slightly exceed the male, although from 16 to 60 years the reverse is true, the hemoglobin of the female averaging close to 15.5 per cent, while in the male it reaches nearly 17 per cent.

A few observations taken from Meyer and Butterfield are given in the table below. They employed the same method as did Williamson and

HEMOGLOBIN CONTENT OF THE BLOOD OF NORMAL AND PATHOLOGICAL SUBJECTS

Subjects	Specific Gravity	Erythrocytes, Million per cu. mm.	Hemoglobin Content of Blood, gm. per 100 c.c.	Color Index
Normal men, av. 7 cases	1.059	4.92	16.60	1.0
Normal women, av. 6 cases	1.057	4.75	15.20	1.0
Pernicious anemia, I	1.040	0.74	3.47	1.5
Pernicious anemia, II	1.035	0.87	3.79	1.3
Secondary anemia	.....	2.43	5.59	0.7
Polycythemia	1.075	....	23.90	...

their figures for normal adults are in substantial agreement with those recorded above. The few pathological data are of interest. In the cases of pernicious anemia it will be noted that the hemoglobin dropped to the low figure of about 3.5 per cent, while in the case of polycythemia it reached 23.9 per cent.

Since the serum proteins, albumin and globulin, vary only to a limited extent, as previously noted, it is apparent that hemoglobin is ordinarily not only the largest but also the most variable factor in the make-up of the total solids. For this reason hemoglobin estimations provide a simple method of securing information regarding the total solid content of the blood. Underhill used the estimation for this purpose to excellent advantage in the treatment of poisoning with lethal war gases. It may be assumed that daily fluctuations in the amount of hemoglobin in the circulating blood are slight and that such fluctuations in the hemoglobin content are due to changes in blood volume. The frequent estimation of the hemoglobin content of the blood in short series of experiments therefore constitutes a simple means of following small changes in blood volume.

There would seem to be no good reason why the clinical estimation of hemoglobin should not be put on a more exact basis, comparable with many of our other chemical blood analyses. Palmer (*b*) has recently described a very simple and accurate method of estimating hemoglobin as carboxyhemoglobin, while Van Slyke's (*c*) method of determining the oxygen capacity of the blood is valuable in furnishing an occasional check on the colorimetric methods and in the preparation of a blood standard. It should also be noted that several recent papers have shown that hemoglobin can be accurately estimated by the acid hematin method of Sahli, provided certain precautions are followed and a good colorimeter employed.

Blood Cells.—The blood cells (erythrocytes, leucocytes and blood plates) are of interest in this connection only in so far as variations in their content affect the chemical composition of the blood as a whole. The figures which are generally given for the erythrocytes of the adult male and female are 5 million per cubic millimeter for the former and 4.5 million for the latter. Values higher than these are not uncommon but the number rarely exceeds six million in perfectly normal individuals. Since the red cells are composed of hemoglobin roughly to the extent of 90 per cent it is apparent that the hemoglobin content, and the total solid content as well, stand in fairly close relationship to the number of red cells. In pernicious anemia the number of cells may be reduced to as small a number as 0.5 million or even less, while in some cases of secondary anemia very low figures are found. Meyer and Butterfield have pointed out that the high color index observed in many cases of pernicious anemia is due to an increase in the oxyhemoglobin content of the red cells (see table on p. 430). In the secondary anemias the color index is frequently lowered, apparently for the reverse reason. As would seem evident from the

hemoglobin table of Williamson above, the red cell count is very high at birth, reaching 7 million in some instances, but drops to a fairly constant level after the sixth to the tenth day. Owing to the diminished oxygen tension at high altitudes the number of red cells is increased to maintain the oxygen carrying capacity of the blood at a normal level, the number being raised to 7 to 9 million in extreme instances. A relative increase in the number of red cells, or relative polycythemia, may occur as result of sweating, diarrhea, etc., while an absolute polycythemia is occasionally encountered, particularly in congenital heart disease and in Osler's disease. The number of leucocytes normally varies between 3,000 and 10,000 per cubic millimeter, although figures between 5,000 and 6,000 are the most often encountered in a fasting condition. The leucocytes are subject to greater physiological variation than the red cells, but considering their much smaller number in comparison with the red cells, these variations have little influence on the chemical composition of the blood as a whole. In the leucemias, however, the leucocyte count may rise to 600,000 and even higher. With such a marked leucocytosis, and consequent leucolysis, the uric acid content of the blood may be greatly increased. Although the blood plates are normally regarded as amounting to from 200,000 to 500,000 per cubic millimeter, on account of their small size, 3 μ, their variation is apparently without influence upon the chemical composition of the blood.

Blood Nitrogen

Total Nitrogen.—The total nitrogen content of perfectly normal blood amounts to somewhat more than 3 per cent. Of this, 99 per cent is derived from the various proteins of the blood, about three-quarters being from the cellular constituents, chiefly the hemoglobin, and one-quarter from the plasma proteins, albumin, globulin and fibrinogen. The hemoglobin is obviously the most important as well as the most variable contributor to the total nitrogen. In pernicious anemia the total nitrogen may be reduced to considerably less than half the normal figure, while in severe nephritis the nitrogen content is frequently very low.

Non-protein Nitrogen.—Although the non-protein nitrogen normally constitutes only about one per cent of the total nitrogen of the blood, nevertheless greater interest is attached at the present time to variations in the bodies which form the non-protein than the protein nitrogen. This is due largely to the fact that the variations in these non-protein constituents give us an insight into some of the processes of anabolism and catabolism. The food nitrogen is carried by the blood to the various tissues and the waste nitrogen to the kidneys, directly or indirectly by the same medium. After a meal containing protein there is a temporary elevation in the non-protein and amino nitrogen of the blood. In diseases of the kidney

there may be at first only a slight rise in the uric acid or urea, although in the terminal stages of the disease there is generally a very marked elevation in all the forms of non-protein nitrogen. The normal range of the various non-protein nitrogenous components is given in the table below. Data are also included indicating the deviations which may occur in gout, interstitial and parenchymatous nephritis and eclampsia.

As will be noted in the table, the normal range for the non-protein nitrogen is given as 25-30 mg. per 100 c.c. of blood. In discussing the question of the normal values for the non-protein nitrogen there are two very important factors which should always be considered, viz., the protein precipitant employed and the proximity to the last meal. The results reported with the original method of Folin and Denis (*f*) are probably a little too low, owing to the use of methyl alcohol as the protein precipitant. Folin and Denis originally obtained figures of 22-26 mg., while Tileston and Comfort found 23-25 mg. with a series of five normal adults in a fasting state, and 26-32 mg. two and a half hours after a heavy protein meal. More satisfactory results are obtained after the trichloracetic acid precipitation of Greenwald (*d*) or use of the tungstic acid reagent recently employed by Folin and Wu. After these methods of precipitation figures close to 30 mg. are generally obtained on a normal individual in the fasting state.

NONPROTEIN NITROGENOUS CONSTITUENTS, MG. TO 100 C.C. OF BLOOD

Constituents	Normal	Gout	Early Interstitial Nephritis	Terminal Interstitial Nephritis	Parenchymatous Nephritis	Eclampsia
Non-protein N.	25–30		30–50	to 350		35–55
Urea N.....	12–15		12–30	300	30–60	7–16
Uric Acid.....	2–3	4–10	3–10	25		3–10
Creatinin	1–2		2–4	35		1–2.5
Creatin	3–7			30		
Amino Acid N.	6–8			30		4–8
Ammonia N...	0.1			1		

The figures for the normal creatin are taken from observations of Denis, those for amino-acid nitrogen from Bock, except in the case of eclampsia, where the observations of Losee and Van Slyke are recorded; other data in eclampsia are from recent observations of Killian. With these exceptions the data are from the writer's observations.

The figures for ammonia are very small, but these figures may be taken as the maximal rather than the minimum values. The very recent observations of Nash and Benedict on the ammonia content of the blood (made on dogs and cats) give figures between 0.03 and 0.2 mg. to 100 c.c.

The origin and rôle which the various non-protein nitrogenous constituents play in metabolism, as well as the ease of kidney secretion, obviously greatly influence the content of these substances in the blood, both normally and pathologically. Folin's classic papers on the composition of urine (for discussion, see Chapter IV) published in 1905, did much to give

us a correct appreciation of the significance of the nitrogenous waste products which find their exit through the kidney. He pointed out that the urea and creatinin stood in marked contrast to each other, since the former was largely exogenous in origin, while the latter was almost entirely of endogenous formation. Uric acid stood in somewhat of an intermediate position, being about half endogenous and half exogenous under ordinary conditions of diet.

Satisfactory interpretations of variations in these non-protein nitrogenous constituents of the blood can scarcely be made without a knowledge of their origin. The following brief statement may be made regarding the formation of these compounds. Urea is formed largely in the liver from the ammonia resulting from the deaminization of amino-acids set free in digestion, but not of immediate use to the animal organism. Uric acid originates as a result of the enzymatic transformation of the amino- and oxypurins, in which various glands of the body participate. Creatinin would appear to be formed in the muscle tissue from creatin.

COMPARATIVE NITROGEN PARTITION OF URINE AND BLOOD IN PER CENT OF TOTAL NON-PROTEIN NITROGEN

Fluid	Uric Acid N	Urea N	Creatinin N	Ammonia N	Rest N
Normal urine	1.5	85	5	4	4.5
Normal blood	2	50	2	0.3	46
Blood in gout and early nephritis ...	6	50	2	0.3	42
Blood in parenchymatous nephritis (nephrosis).....	2	55	2	0.3	40
Blood in terminal interstitial nephritis.	2 to 3	75	2.5	0.5	20

It is of interest to compare the partition of the non-protein nitrogenous constituents in the blood with similar partition in the urine. (See above.) Upon the ordinary mixed diet their approximate distribution in the urine is 85 per cent urea N, 1.5 per cent uric acid N, 5 per cent creatinin N, 4 per cent ammonia N and 4.5 per cent undetermined N. It is quite natural to expect a somewhat similar relationship in the non-protein nitrogenous constituents of the blood, but the above table discloses quite a different distribution. It will be noted that even in normal blood the percentage of uric acid nitrogen is greater, if anything, than in the urine, while the urea is definitely lower, the contrast with the uric acid in the case of the creatinin and ammonia being even more marked. As Folin and Denis have pointed out, the human kidney removes the creatinin from the blood with remarkable ease and certainty, the completeness of the creatinin excretion being exceeded only by the still more complete removal of the ammonium salts. The striking difference between the ability to excrete uric acid on the one hand, and urea and creatinin on the other, is

brought out from an examination of the normal concentration of the blood and urine. Judging from their comparative composition, the kidney normally concentrates the creatinin 100 times, the urea 80 times, but the uric acid only 20 times. Myers, Fine and Lough have pointed out that as the permeability of the kidney is lowered in conditions of renal insufficiency, this becomes evident in the blood, first by a retention of uric acid, later by that of urea, and lastly by that of creatinin, indicating that creatinin is the most readily eliminated of these three nitrogenous waste products, and uric acid the most difficultly eliminated, with urea standing in an intermediate position.

Urea.—As indicated in the table above on non-protein nitrogenous constituents the blood urea of a strictly normal individual taken in the morning before breakfast appears to fall within the comparatively narrow limits of 12-15 mg. urea nitrogen per 100 c.c. of blood. Occasionally figures outside of the limits may be observed such as 10-18 mg., but figures above 20 mg. can ordinarily be regarded as pathological. These statements apply only to normal individuals on moderate protein diets where the blood has been taken in the morning before breakfast. As Tileston and Comfort, and Addis and Watanabe have shown, high protein diets may considerably raise these figures, especially in certain individuals, while Folin, Denis and Seymour have conclusively shown that lowering the level of protein metabolism serves to reduce the non-protein and urea nitrogen of the blood in mild cases of chronic interstitial nephritis.

Since urea is the chief component of the non-protein nitrogen, and since its estimation is considerably simpler than that of the non-protein nitrogen, attention will be directed especially to the urea. Mosenthal and Hiller have made a careful study of the relation of the urea to the non-protein nitrogen in disease. They point out that the selective action of the kidney maintains the urea nitrogen at a level of 50 per cent or less of the total non-protein nitrogen of the blood, but that an impairment of renal function, even of very slight degree, may result in an increase of the percentage of urea nitrogen. In advanced cases this may be even higher than the 75 per cent given in the preceding table.

To give a comparative idea of the values observed for urea nitrogen in various pathological conditions, illustrative findings are given for a number of different conditions in the table below taken from a recent paper by the writer, the data being from actual cases. As will be noted, the conditions in which nitrogen retention may occur are quite numerous. Marked urea retention may occur not only in the terminal stages of chronic interstitial nephritis, but also in such conditions as bichlorid poisoning and double polycystic kidney, and in some cases of acute nephritis. In parenchymatous nephritis the findings are comparatively low. Relatively high figures are frequently noted in malignancy, pneumonia, intestinal obstruction, lead poisoning, and sometimes in syphilis and cardiac condi-

Conditions with Significant Urea Nitrogen Findings

Case	Mg. to 100 c.c. of Blood			Diagnosis
	Uric Acid	Urea N	Creatinin	
1	15.0	240	33.3	Bichlorid poisoning
2	4.5	75	8.5	Double polycystic kidney
3	14.3	263	22.2	Terminal chronic interstitial nephritis
4	9.5	25	2.5	Early chronic interstitial nephritis; died 3 years later
5	8.3	72	3.2	Chronic diffuse nephritis; syphilis
6	2.3	28	1.9	Chronic parenchymatous nephritis
7	11.4	106	6.1	Severe acute nephritis; recovery
8	....	50	2.5	Mild acute nephritis
9	9.7	58	3.4	General carcinomatosis
10	5.5	24	3.1	Carcinoma of larynx
11	9.0	46	3.3	Severe pneumonia; recovery
12	...	43	2.9	Syphilis
13	5.5	44	3.3	Intestinal obstruction
14	...	24	2.5	Gastric ulcer
15	3.3	20	2.0	Duodenal ulcer
16	7.2	18	2.2	Prostatic obstruction
17	...	14	2.9	Myocarditis
18	6.0	18	2.2	Diabetes of long standing
19	8.4	12	2.9	Gout
20	6.8	7	2.2	Eclampsia

tions, although in the last mentioned this is probably due to renal complications. In uncomplicated cases of prostatic obstruction the findings do not appear to much exceed 20 mg. urea nitrogen. A slight retention is frequently noted in gastric and duodenal ulcer, possibly for the same reason that retention is found in intestinal obstruction. Advanced cases of diabetes frequently show definitely high figures, apparently due in some instances to the high protein diet, in others to a complicating nephritis. The fact that a normal urea is associated with a high uric acid is of practical value in cases of gout not complicated by nephritis. In normal pregnancy, the findings for urea nitrogen are, strangely enough, subnormal, figures between 5 and 9 having been observed. In eclampsia the urea is generally subnormal, but the non-protein nitrogen is increased and the uric acid is generally quite high.

Since urea is largely of exogenous origin, while creatinin is endogenous, it is subject to much greater variation, especially under dietary influences. It is of less prognostic value than the creatinin in advanced cases of nephritis, but a much better guide as to the value of the treatment. In cases of prostatic obstruction the urea is an excellent preoperative prognostic test, much better than the creatinin, for the reason that cases showing creatinin retention already show sufficient urea retention to make them very poor risks. The renal factor can be disregarded when the urea nitrogen is 20 mg. or under, the patient operated on with caution between 20 and 30, while with figures over 30 the outlook is un-

favorable. Nephritis in children does not so quickly result in urea retention as in the adult. On this account it is an especially helpful prognostic test in the nephritis occurring in early life.

Uric Acid.—No accurate figures on the uric acid content of normal human blood were available until the introduction of the colorimetric method of Folin and Denis (*e*) in 1913. In a series of unselected cases Folin and Denis (*b*) found between 1 and 3 mg. to 100 c.c. of blood, the average being close to 2 mg. Although the accuracy of the method of estimating uric acid has been considerably improved, still the figures which are now regarded as normal for the blood uric acid differ very little from those originally reported by Folin and Denis. Healthy adults most often yield values between 2 and 3 mg. per 100 c.c. of blood, but figures as low as 1 mg. and as high as 3.5 mg. may be encountered in strictly normal individuals, the difference probably depending in part upon dietary factors. High blood uric acids must obviously depend upon either an increased formation or a decreased elimination.

In leucemia the first factor accounts for the increase, but high uric acids in most other conditions find a probable explanation on the latter basis. Among these may be mentioned nephritis, acute and chronic (but not parenchymatous), arterial hypertension, lead poisoning, bichlorid poisoning, malignancy, acute infections, especially pneumonia, gout and apparently some cases of non-gouty arthritis. Miscellaneous cases illustrating the uric acid findings in many of these conditions are given in the urea table above. Sedgwick and Kingsbury have made the interesting observation that the blood uric acid is high during the first three or four days of life, in harmony with the high uric acid excretion during that period.

That the uric acid content of the blood was increased in gout was recognized more than seventy years ago by Sir A. B. Garrod. He put the subject of the uric acid content of the blood on a definite basis when he identified this substance in the blood of patients suffering from gout, and showed that whereas uric acid was normally present in blood only in traces, it was definitely increased not only in gout, but also in certain cases of nephritis. He further showed that there is no increase in the blood uric acid in rheumatism, such as is found in gout, and used this as a point of differential diagnosis. No noteworthy advance in this subject was made until the advent of the colorimetric method of Folin and Denis previously referred to.

In their original paper Folin and Denis (*b*) found practically no elevation of the uric acid in a series of eleven nephritic bloods with only moderate nitrogen retention, but later they reported data on cases of advanced nephritis in some of which very high values were obtained, up to 10 mg. These latter observations were confirmed by Myers and Fine (*g*), who noted very high figures for uric acid in several cases of terminal interstitial nephritis. In one case the uric acid reached the enormous figure of 27 mg.

shortly before death, while in several cases figures as high as 15 mg. were observed, values much higher than any noted in gout. It is perfectly logical to expect that high figures would be found in the last stages of chronic interstitial nephritis, with the consequent accumulation of all the waste products of nitrogenous metabolism. That the retention of uric acid in nephritis results in a fairly even distribution of this substance in the various body tissues has been shown by Fine (*a*) in tissues obtained at autopsy. The distribution, however, is not quite as uniform as in the case of the urea or even the creatinin, a fact which might be expected from their physical properties.

In 1916 Myers, Fine and Lough called attention to the fact that very high figures for uric acid may be noted, not only in cases of advanced interstitial nephritis, but also in the very early stages of the disease, before a retention of either the urea or creatinin had taken place. It was suggested that, when symptoms of gout were absent, a high blood uric acid might be a valuable early diagnostic sign of nephritis, possibly earlier evidence of renal impairment of an interstitial type than the classic tests of proteinuria and cylinduria. This point is well illustrated by the staircase table on page 439, taken from Chace and Myers. As a result of a recent study of this question Baumann, Hansmann, Davis and Stevens conclude that the uric acid concentration of the blood is a delicate, if not the most delicate, index of renal function at our disposal.

Owing to the fact that the tophi found in gout have long been recognized to contain deposits of sodium urate, it is quite natural that the uric acid content of the blood in this condition should possess a special interest. Following the investigations of Folin and Denis a number of different workers took up a study of this question. Among these in particular should be mentioned Pratt, Fine and their coworkers. From the normal variations of from 2 to 3 mg. to 100 c.c. of blood, the uric acid may be increased to as much as from 4 to 9 mg. in gout, but it does not follow that these uric acid accumulations are infallible signs of gout, since, as noted above, similar uric acid figures may be found in nephritis. We may conclude, however, that gout is almost invariably associated with an increased uric acid content of the blood and therefore a high uric acid blood may be of considerable diagnostic value in cases of gouty arthritis, in which tophi containing sodium urate are not already present.

High figures for the blood uric acid may be considerably reduced in many cases, where appreciable urea retention does not exist, by the use of purin free diets. Such diets will not, as a rule, equally influence the blood uric acid in gout, although appreciably lowering the initial figures.

It is of considerable interest in this connection that salicylic acid, phenylcinchoninic acid (cinchophen) and certain of their derivatives have recently been shown to have a marked influence upon the elimination of uric acid and upon the uric acid content of the blood. In many cases mod-

URIC ACID, UREA NITROGEN AND CREATININ OF BLOOD IN CHRONIC NEPHRITIS

Date 1915–16	Case	Age	Sex	Diagnosis	Condition	Mg. per 100 c.c. of Blood			Phthalein 2 Hrs., Per Cent	Systolic Blood Pressure	Urine	
						Uric Acid	Urea N	Creatinin			Albumin	Casts
I												
9/17	H. L.	23	M	Pulmonary tuberculosis	Unchanged	6.5	16	2.7	58	130	++	+
8/10	E. H	41	M	Pericarditis	Unchanged	5.6	13	2.1	45	150	—	—
10/12	F. D.	45	M	Interstitial nephritis	Unchanged	5.5	12	2.5	37	185	—	+
3/ 6	B. D.	35	F	Diffuse nephritis	Unchanged	9.6	19	2.4	45	175	+	+
II												
8/11	J. J.	65	M	Early interstitial nephritis	Unchanged	9.5	25	2.5	13	185	+	+
7/21	D. S.	56	M	Early interstitial nephritis	Unchanged	6.6	24	3.3	26	185	—	+
9/21	D. D.	52	M	Early interstitial nephritis	Unchanged	8.7	20	3.6	20	100	+	+
8/ 3	C. M.	54	M	Early interstitial nephritis	Unchanged	6.3	31	2.0	23	150	—	—
III												
1/ 6	L. P.	57	M	Moderately severe chronic interstitial nephritis	Improved	8.0	80	4.8	0	240	++	++
3/ 1						4.9	17	2.9	10	170		
4/23	J. P.	34	M	Moderately severe chronic diffuse nephritis	Improved	8.3	72	3.2	25	238	+++	++
5/21						5.3	21	1.9	43	145		
1/15	W. C.	49	M	Moderately severe chronic diffuse nephritis	Improved	9.5	44	3.5	38	210	++	++
1/28						2.5	19	1.9	52	120		
IV												
4/11	E. C.	50	F	Typical fatal case of chronic interstitial nephritis	Died	22.4	236	16.7	0	210	++	Pus
3/23	T. D.	34	M	Typical fatal case of chronic interstitial nephritis	Died	15.0	240	20.5	2-3	225	++	+
1/25	S. H.	37	M	Typical fatal case of chronic interstitial nephritis	Died	14.3	263	22.2	0	220	++	+
4/15	J. W.	34	M	Typical fatal case of chronic interstitial nephritis	Died	8.7	144	11.0	Trace	225	+	+

erate doses of cinchophen will reduce a uric acid content of 5 or 6 mg. to a mere trace in a comparatively few hours. If long continued, however, the drug loses this influence. This uric acid eliminating effect appears to be quite independent of the marked analgesic effect of these drugs.

Creatinin.—Until the advent of Folin's colorimetric method for the estimation of creatinin in urine in 1904, we possessed no reliable information regarding this interesting nitrogenous waste product. Folin was the first to show that the amount of creatinin excreted in the urine by a normal individual on a meat free diet is quite independent of either the amount of protein in the food or of the total nitrogen in the urine, the amount excreted from day to day being practically constant for each individual, thus pointing conclusively to its endogenous origin. In 1914 Folin (*f*) applied his colorimetric method, slightly modified, to the estimation of creatinin in blood, and Folin and Denis (*g*) presented some quite extensive data on the subject. Almost simultaneously Neubauer reported an observation on a case of "uremia," while Myers and Fine (*g*) presented several analyses on two cases of nephritis showing marked retention of creatinin.

For perfectly normal individuals the creatinin of the blood amounts to 1 to 2 mg. per 100 c.c., the findings for the strictly normal being nearer 1 than 2 mg. This statement should probably be made with some reservation as the method does not appear to be entirely adequate for the determination. It is quite possible that the actual content of creatinin may not be much more than 0.5 mg., the remainder being due to the interference of other substances in the color reaction. The figures obtainable with present methods are comparable, however, and serve as a satisfactory base line. The importance of this source of error would appear to decrease proportionately with a rise in the creatinin content of the blood, so that the absolute accuracy of the estimation is much greater with pathologic than normal values.

As soon as one passes to hospital patients values higher than 1 to 2 mg. are found. Although the great majority of cases without renal involvement show creatinin figures on the whole blood below 2.5 mg. per 100 c.c., occasionally figures as high as 3.5 mg. are encountered that are not readily explained. It may be noted, however, that a slight retention of creatinin (figures between 3 and 4 mg.) occurs in syphilis, certain heart conditions, sometimes in fevers, and in some cases of advanced diabetes. Creatinin figures above 3.5 mg. are almost invariably accompanied by an appreciable urea retention and this is generally true of those above 3 mg. Many of the cases below 4 mg. show improvement, but with over 4 mg. the reverse is the case. It would appear from this that an appreciable retention of creatinin, i. e., over 4 mg., does not occur until the activity of the kidney is greatly impaired. That such should be the case is quite natural to expect, since creatinin is normally the most readily eliminated

of the three nitrogenous waste products, uric acid, urea, and creatinin (see staircase table on page 439).

In various studies on nitrogen retention by Myers and associates it was soon noted that the creatinin of the blood was appreciably increased only after considerable retention of urea had already taken place and the nephritis was rather far advanced. It was further observed that those cases in which the creatinin had risen above 5 mg. per 100 c.c. of blood rarely showed any marked improvement, and almost invariably died within a comparatively limited time. The only exceptions were cases where the retention was due to some acute renal condition. In a recent paper Myers and Killian (*b*) have discussed in detail the observations on a series of 100 nephritics with high creatinin findings, while more recently Myers has again reviewed the general subject. It may be stated that of 85 cases having over 5 mg. of creatinin, all the cases, with three exceptions, are known to be dead. Most of these cases lived from 1 week to 3 months although there were three cases that lived 1, 2 and 3 years respectively. Of the three exceptions two were acute cases that recovered, while one was followed for only a short period. Among the cases having very high blood creatinins there were many who were able to be up and about and some who showed considerable clinical improvement. In these cases the blood creatinin gave a particularly good insight into the true nature of the condition.

The amount of the increase of the creatinin of the blood should be a safer index of the decrease in the permeability of the kidney than the urea, for the reason that creatinin on a meat free diet is entirely endogenous in origin and its formation (and elimination normally) very constant. Urea, on the other hand, is largely exogenous under normal conditions and its formation consequently subject to greater fluctuation. For this reason it must be evident that a lowered nitrogen intake may reduce the work of the kidney in eliminating urea, but cannot affect the creatinin to any extent. Apparently the kidney is never able to overcome the handicap of a high creatinin accumulation. It would seem that creatinin, being almost exclusively of endogenous origin, furnishes a most satisfactory criterion as to the deficiency in the excretory power of the kidneys and a most reliable means of following the terminal course of the disease, though it should be noted that urea, being largely of exogenous origin, is more readily influenced by dietary changes, and therefore constitutes a more sensitive index of the response to treatment.

Creatin.—The methods of estimating the blood creatin are considerably less satisfactory than those for creatinin. Figures obtained with the original Folin method were apparently too high. Recent methods and observations of Denis(*b*) and Folin and Wu give the normal creatin content of blood as from 3 to 7 mg., with an average of about 5 mg. The

amount does not appear to be increased except in terminal nephritis with marked nitrogen retention, when values as high as 30 mg. may be attained. According to Hunter and Campbell (*b*) the average creatin content of the corpuscles lies roughly between 6 and 9 mg. per 100 c.c., while that of the plasma is not more than 0.4 to 0.6, the blood as a whole containing about 3 mg., and slightly higher figures being found in females than males. According to these investigators there is a distinct correspondence between increase of plasma creatin and the appearance of creatin in the urine; but whether the plasma, in the absence of creatinuria, is creatin-free or whether there exists a threshold for creatin excretion, has not been positively determined.

Amino-Acids.—That the amino-acids formed in proteolytic digestion are taken up directly by the blood was first clearly shown by Van Slyke and Meyer (*a*), employing Van Slyke's method for the determination. This had been made probable from results obtained for the non-protein nitrogen of the blood by Folin and Denis shortly before, but the work of Van Slyke and Meyer conclusively proved this point, thus definitely settling one of the long disputed questions of protein absorption. They found, for example, that whereas the amino-acid nitrogen of a normal fasting dog amounted to 4 to 5 mg. per 100 c.c. of blood, it was increased to 9 to 10 mg. after a heavy protein meal.

Comparatively few data are available for the amino-acid nitrogen content of human blood. The normal content of amino nitrogen may be given as 4 to 8 mg., with an average close to 5 mg., per 100 c.c. of blood. In a series of sixty practically normal subjects Hammett (*c*) found the amino nitrogen to be relatively constant with an average of 4.9 and variations of 3.1 to 7.2 mg. per 100 c.c. of blood. Bock has reported analyses on a series of miscellaneous pathological cases. He failed to find any noteworthy deviations from the normal except in severe nephritis, where in several cases figures exceeding 10 mg. and in one instance 30 mg. was reached. In general the findings of Hammett and Bock harmonize very well, though the figures of Hammett average slightly lower, possibly due to the fact that he used tungstic acid as the protein precipitant, while Bock employed trichloracetic acid.

Ammonia.—According to the recent observations of Nash and Benedict, the ammonia nitrogen content of the blood (of dogs and cats) under normal and various experimental conditions is close to 0.1 mg. per 100 c.c. They express the view that the urea of the blood is the probable precursor of the urinary ammonia, and that the kidney is the seat of this transformation.

Rest Nitrogen.—The amount of undetermined nitrogen present in protein-free blood filtrates appears always to be very large. In the table on page 434 the normal rest nitrogen was given as 45 per cent of the total non-protein nitrogen. Here the creatin and amino-acid nitrogen were included. If deductions of 4 per cent are made for the creatin nitrogen

and 14 per cent for the amino-acid nitrogen, 28 per cent of the total non-protein nitrogen still remains unaccounted for. With the rise in the urea nitrogen that occurs in many cases of nephritis with marked nitrogen retention there is a corresponding decline in the percentage of the rest nitrogen, indicating that the actual amount of the rest nitrogen remains fairly constant under abnormal conditions. As pointed out by Hammett, there is, however, considerable variation in the amount of the rest nitrogen of practically normal individuals. He found variations of 4 to 18 mg. with an average of 11 mg. to 100 c.c. in sixty cases. These figures represent the difference between the non-protein nitrogen and the sum total of the urea, uric acid, creatinin, creatin and amino-acid nitrogen. While our methods are not sufficiently accurate to make the findings for the rest nitrogen reliable, still they do indicate that this fraction is quite large. At the present time we possess no very good information as to the nature of this material in human blood, although it would seem possible from the experimental work of Whipple and Van Slyke on proteose intoxication that a large part of this nitrogen was derived from peptids. From the work of Abel we also have reason to believe that traces of proteoses are present.

Blood Sugar.—A sugarlike substance was first recognized in the blood in a case of diabetes by Dobson in 1775, but it was not until seventy years later that its presence in normal blood was discovered by the noted French physiologist, Claude Bernard. By means of his sugar piqûre Bernard first noted the connection between hyperglycemia and glycosuria (glycuresis). It remained for Lewis and Benedict in 1913 to introduce a colorimetric method for blood sugar estimation so simple that it could be readily employed for clinical as well as scientific purposes. Earlier in the same year Bang had described a very ingenious method requiring only two to three drops of blood, but the fact that it was a gravimetric-volumetric procedure precluded any very extensive clinical application. Stimulated by these methods, and several others since devised, many studies dealing with the sugar of the blood have recently appeared. Previous to the introduction of these simple methods, however, Bang (*d*) had written a very interesting monograph under the title "Der Blutzucker," while Macleod(*b*) had discussed the subject of diabetes almost entirely upon the basis of experimental observations on the blood sugar.

If we may rely upon the findings with the Benedict method, the blood sugar of the normal human subject falls somewhere between 0.09 and 0.12 per cent, on the average being about 0.10 per cent. Depending upon the method which is employed for the estimation, one may obtain figures differing as much as 0.02 per cent in the normal bood, while with pathological bloods the differences, as shown by Höst and Hatlehol, may be somewhat greater. Slightly higher figures appear to be obtained by the picric acid method of Benedict in its various modifications than by most

of the other methods. That the reducing power of the blood is due in large part to glucose seems certain, although the various methods appear to be influenced by other reducing substances. Of the known interfering substances creatinin is the most often mentioned. In normal blood, however, it probably does not introduce an error of more than 2 or 3 per cent. Although the question of the actual content of glucose in normal blood is one of great theoretical interest and importance, the figures obtained by the various methods differ so little relative to the variations which occur in disease that the question of the method scarcely enters into a discussion of blood sugar findings in disease.

The figure of 0.10 per cent for normal individuals given above applies to observations made in the morning previous to the intake of any carbohydrate. After a meal rich in carbohydrate there may be an appreciable rise in the sugar content of the blood, 0.12 to 0.14 per cent, while after the intake of even moderately large amounts of glucose, the hyperglycemia, 0.15 to 0.16 per cent, may be sufficient to induce a slight temporary (glycosuria) glycuresis. The great majority of hospital cases show practically normal figures for blood sugar, although occasionally figures of 0.12 to 0.15 per cent are encountered that are not readily explained.

Conditions of hyperglycemia are much more common and of greater clinical interest than those of hypoglycemia, owing primarily to the fact that diabetes belongs to the former group. Among other conditions which frequently show moderate hyperglycemia are pancreatic disease, nephritis and hyperthyroidism. Hypoendocrin function would appear to result in hypoglycemia, and comparatively low blood sugars have been observed in myxedema, cretinism, Addison's disease, pituitary disease and other less clearly defined endocrin conditions such as muscular dystrophy.

All forms of glycosuria are accompanied by hyperglycemia, if we except the glycosuria produced by such substances as phlorhizin and uranium, and the analogous condition, "renal diabetes." In mild cases of diabetes the hyperglycemia is not excessive, generally 0.2 to 0.3 per cent, although in severe cases figures up to and even above 1.0 per cent have been obtained. The normal threshold of sugar excretion (i. e., the point of glycuresis) is about 0.16 to 0.18 per cent. With blood sugar concentrations of 0.15 to 0.20 per cent the appearance of sugar in the urine is apparently dependent on whether or not diuresis exists, glycosuria appearing especially in the latter case. When the threshold point has been passed, however, the overflow of sugar into the urine may continue until the concentration in the blood has fallen nearly to normal. Mild cases of diabetes usually have a normal threshold, although some severe cases apparently have a lowered threshold, increasing the severity of the condition. Ordinarily in the early stages of the disease there is a fairly direct relationship between the hyperglycemia and glycosuria. In the later stages of the disease, however, cases are frequently encountered with marked hyperglycemia and

only slight glycosuria, showing that the threshold point has been raised, apparently due in many instances to an accompanying nephritis. The cause of glycosuria in "renal diabetes" is obviously due to the reverse condition, viz., a threshold point below the level of the normal blood sugar.

A simple method of estimating the diastatic activity of the blood has been described by Myers and Killian (*a*) who have called attention to the fact that conditions of hyperglycemia are associated with an increased diastatic activity and have suggested that this might be the important factor in the production of the hyperglycemia in both diabetes and nephritis. The increase in the diastase of the blood in nephritis finds probable explanation in the decreased excretion of diastase in the urine, now well known in this condition, although a satisfactory explanation of the increased activity in diabetes is not so readily given. So-called alimentary glycosuria is apparently due to an increased activity on the part of this diastatic ferment, thus impairing the body's power to store glycogen. Hyperfunction on the part of the ductless glands, hyperthyroidism for example, appears to result in an increase in the blood diastase, while hypofunction seems to have the reverse effect.

Blood Lipoids

Material contributions to our knowledge of the blood lipoids and fat metabolism have been made during the past ten years. The blood lipoids comprise (1) the true fats—glycerids of the fatty acids; (2) the phosphatids—lecithin, cephalin, etc., ordinarily called lecithin, and (3) cholesterol with its fatty acid esters. Although these substances were originally grouped together on account of similar solvent properties, it would now appear that they are closely connected in metabolism.

Bloor (*d*) has carried out experiments which support the older conception of fat digestion, i. e., the food fat is saponified in the intestine, absorbed in water soluble form as soaps and glycerol, resynthesized by the intestinal cells, and passed into the chyle and thence to the blood as neutral fat suspended in the plasma in a very fine condition. About 60 per cent of the food fat has actually been accounted for in the chyle in this way and this figure is probably low. The remaining smaller quantity is generally assumed to be absorbed directly into the blood stream by way of the intestinal capillaries.

In a study of the blood lipoids during fat assimilation, Bloor (*e*) has observed that (1) the total fatty acids increase in both plasma and corpuscles but the increase is generally more marked in the corpuscles; (2) lecithin increases greatly in the corpuscles, but only slightly in the plasma; (3) no definite change takes place in the quantity of cholesterol and (4) a fairly constant relationship exists between the total fatty acids and lecithin of

the whole blood and corpuscles. From this Bloor suggests: (a) that the blood corpuscles take up the fat from the plasma and transform it into lecithin; (b) that most, if not all, of the absorbed fat is so transformed; and therefore (c) that lecithin is an intermediate step in the metabolism of the fats.

Since the question of the blood lipoids has been very carefully considered by Bloor in a series of papers, an abbreviated table showing his average normal findings and three illustrative pathological (extremely severe) cases is given below. It will be noted in the data on the normals that the *lecithin* content of the corpuscles is approximately double that of the plasma, while the *cholesterol* and *total fatty acid* values are almost always lower in the corpuscles than in the plasma. The value for lecithin in the corpuscles is generally about twice that of the cholesterol, while in the plasma their values are nearly equal. According to Bloor the ratio between these constituents is quite constant in normal blood (especially plasma) and remains so in most of the pathological samples, suggesting a definite relationship between these constituents, and making it probable that cholesterol (as its esters?) has a part in fat metabolism.

The most characteristic feature of pathological conditions is the increase of total fatty acids and fat both in plasma and corpuscles, and the decrease of lecithin in the plasma. Since the fat is probably to be regarded as the inactive form of the body lipoids, the form in which they are stored and the lecithin as the first step in the utilization, an undue accumulation of fat or a notably decreased value for lecithin, probably indicates a diminished activity of the fat metabolism.

In severe diabetes the blood lipoids are all greatly increased but the ratios between those constituents are practically normal. The fact that the cholesterol increases parallel with the fat in diabetic blood, even in severe lipemia, supports the view that probably cholesterol plays an important part in fat metabolism. Since cholesterol may be rather simply estimated it affords a practical method of gauging the severity of diabetic lipemia. In mild diabetes the blood lipoids may be practically normal.

While there is no certain evidence that the abnormalities in the blood lipoids are responsible for anemia, the low values for cholesterol, which is an antihemolytic substance, and the high fat fraction, which may indicate the presence of abnormal amounts of hemolytic lipoids in the blood, are possible causative factors.

According to Bloor (*f*) the changes in the blood lipoids in severe nephritis are a high fat in the plasma and corpuscles and high lecithin in the corpuscles. These abnormalities are the same as are found in alimentary lipemia and may be regarded as the result of a retarded assimilation of fat in blood, due possibly to a metabolic disturbance brought about by a lowered alkali reserve of the blood and tissues.

LIPOID CONTENT OF NORMAL AND PATHOLOGICAL HUMAN BLOODS

Condition	Corpuscles	Total Fatty Acids			Lecithin			Cholesterol			Fat		Total Plasma Lipoids
		Whole Blood	Plasma	Corpuscles	Whole Blood	Plasma	Corpuscles	Whole Blood	Plasma	Corpuscles	Plasma	Corpuscles	
Average, 20 normal men.....	43	0.36	0.38	0.35	0.30	0.22	0.40	0.21	0.22	0.19	0.14	0.07	0.67
Average, 16 normal women.....	38	0.36	0.40	0.29	0.29	0.19	0.44	0.23	0.24	0.21	0.16	0.00	0.69
Diabetic lipemia, extreme.....	29	6.10	8.13		0.46	0.50	0.40	1.26	1.67	0.25	8.17		11.20
Pernicious anemia.....	10	0.37	0.37	0.37	0.16	0.14	0.34	0.16	0.16	0.16	0.21	0.13	0.57
Uremia, death.....	13	0.63	0.63	0.63	0.25	0.18	0.70	0.19	0.18	0.25	0.42	0.13	0.85

The value "total fatty acids" is obtained by subtracting the value for cholesterol from that of "total fat" as determined. The value "lecithin" is obtained by multiplying the value obtained for phosphoric acid (lipoid) by 8. "Fat" in the plasma is obtained by subtracting the fatty acids combined as "lecithin" (0.70 × the lecithin value) together with those combined as cholesterol esters (0.48 × the cholesterol value) from the "total fatty acids" and multiplying the result by 1.05; in the corpuscles, by subtracting the fatty acids combined as "lecithin" from the "total fatty acids" and multiplying by 1.05 as before. The values given for the corpuscles are calculated from those of the whole blood and plasma, taking into account the percentage of the corpuscles. The value "total plasma lipoids" is obtained by adding together the values for "lecithin," cholesterol (including esters), and "fat."

For the different lipoid constituents the following statements may be made:

Total Fat (*Plasma Lipoids*).—Normally the "total fat" content of the blood plasma amounts to 0.6 to 0.7 per cent, but in severe diabetes figures as high as 26 per cent have been observed. In diabetic cases of ordinary severity, however, the figures amount to about 1.5 per cent. Nephritics frequently show a moderately increased fat although the figures rarely reach 1 per cent.

Lecithin.—The normal figures for lecithin may be given in round numbers as 0.2 per cent for the plasma, 0.3 per cent for whole blood and 0.4 per cent for the corpuscles. In diabetes there is an increase in the lecithin of both the corpuscles and the plasma, although in severe lipemia it is more noticeable in the latter. In anemia the lecithin of the plasma in particular is lowered, while in nephritis there is a noteworthy increase in the corpuscles.

Cholesterol.—With the method of Bloor comparatively high figures for cholesterol are obtained, normals of 0.20 to 0.24 per cent on whole blood, with slightly higher figures for the plasma. Figures for whole blood obtained with most of the other methods described in the literature are 0.14 to 0.17 per cent for normal individuals. Figures obtained with Bloor's most recent method are probably too high. The distribution of cholesterol in blood is well illustrated in the following table taken from Grigaut, who was the first to suggest and use a colorimetric method for the estimation of cholesterol.

Condition	Cholesterol in Per Cent		
	Plasma	Whole Blood	Corpuscles
1. Normal man	0.168	0.159	0.141
2. Normal man	0.170	0.150	0.130
3. Normal woman	0.170	0.168	0.171
4. Normal woman	0.175	0.165	0.140
5. Carcinoma of the pancreas with jaundice	0.068	0.105	0.110
6. Pneumonia	0.098	0.110	0.150
7. Carcinoma of the liver with jaundice	0.228	0.198	0.170
8. Diabetes	0.246	0.201	0.137
9. Cholelithiasis	0.270	0.225	0.180
10. Nephritis	0.450	0.285	0.150
11. Nephritis	0.514	0.264	0.135
12. Carcinoma of the pancreas with jaundice	0.840	0.540	0.195

In general it may be stated that hypercholesterolemia is found in arteriosclerosis, nephritis, diabetes (especially with acidosis), obstructive jaundice, in many cases of cholelithiasis, in certain skin diseases, in the early stages of malignant tumors, and in pregnancy. The chief condition in which low values are found is anemia.

As pointed out above cholesterol constitutes an excellent index of the degree of lipemia in diabetes. The decrease in this antihemolytic substance in the plasma in anemia would appear to be of considerable significance.

That cholesterol is partly present in the blood as an ester (fat) has long been recognized. Bloor and Knudson have found that in whole blood the average percentage of cholesterol in combination as esters is about 33.5 per cent, and in the plasma 58 per cent of the total cholesterol.

Acetone Bodies

Owing to the importance which the acetone bodies hold in the acidosis, or more specifically the ketosis, occurring particularly in diabetes the quantities of these substances—*acetone, aceto-acetic acid and β-hydroxybutyric acid*—present in normal and pathological human blood is of considerable interest. Quite recently methods have been described by Marriott, (*a*) and by Van Slyke and Fitz for their estimation in blood. Since acetone is very diffusible it is natural to expect that it should be fairly evenly distributed in the various body fluids, such as the blood and spinal fluid. The concentration in the urine, however, is considerably greater than that in the blood. The amount of the β-hydroxybutyric acid present in both blood and urine is ordinarily in excess of the combined acetone-aceto-acetic acid fraction, often exceeding the latter by two or three times.

According to Van Slyke and Fitz the total acetone bodies of the blood normally amount to 1.3 to 2.6 mg. to 100 c.c. calculated as acetone, while in diabetes as much as 350 mg. have been observed, although patients under ordinarily good control show 10 to 40 mg. Allen, Stillman and Fitz state that there appears to be no constant relation between the plasma alkali and the plasma acetone in diabetes. The acetone bodies may rise greatly even after the carbon dioxid combining power of the blood has been considerably raised by the administration of alkali, and death ensue. The acetone bodies in the blood of children have been studied by Moore. He found in a fairly large series of normal children, that the acetone plus aceto-acetic acid calculated as acetone averages 2.4 mg. to 100 c.c., while the β-hydroxybutyric acid as acetone amounted to 3.9 mg., a total of 6.3 mg. In one case of ileocolitis with acetonuria the total acetone bodies rose to 183 mg. per 100 c.c. shortly before death. Moore states that in a few cases showing acidosis clinically, the acetone of the blood has been found sufficient to account for the acidosis. From a study of the acetone bodies of the blood following ether anesthesia Short concludes that the acetone bodies are not formed promptly enough to account for the decreased plasma bicarbonate.

Mineral Constituents

Sodium.—Comparatively few figures are available for the sodium content of blood. Macallum gives the normal range of figures for normal human plasma as 220 to 316 mg. per 100 c.c., while more recently Kramer has found in adults and children 280 to 310 mg. per 100 c.c. of serum. Greenwald has obtained quite similar figures for dog serum. It has long been recognized that sodium was found chiefly in the body fluids, while potassium was a constituent principally of the cellular tissue. As might be expected, therefore, sodium is found chiefly in the blood plasma, and potassium in the corpuscles. Nothing of special importance is known regarding pathological variations in the sodium content of the blood.

Potassium.—Although the information available at present concerning the potassium content of blood is somewhat limited, considerably more is known than in the case of sodium. Some years ago Abderhalden reported analyses of the blood of different animals. The figures obtained for potassium are of considerable interest. In the dog and cat practically identical figures were found for the serum and whole blood. This amounted to about 22 mg. per 100 c.c., which is almost the exact amount found in the serum of the various animals examined. In the ox, sheep and goat the figures for the whole blood were about one and one-half times that of the serum, while in the horse, pig and rabbit the potassium concentration of the whole blood was about ten times that of the serum.

The potassium content of human blood has recently been considered by Macallum(*c*), Greenwald(*h*), Kramer, and Myers and Short, who are in close agreement that the potassium of normal human blood serum or plasma is a relatively constant quantity and amounts to close to 20 mg. K per 100 c.c. Kramer has suggested a normal range of 16 to 22 mg. to 100 c.c. The potassium content of whole blood depends in large measure upon the cell content, but appears to vary somewhere between 150 and 250 mg. to 100 c.c. in the normal human subject. In primary and secondary anemia the amount may obviously be very low. Pathologically, the potassium content of the serum or plasma is of greater interest. It has been suggested by Smillie that uremic symptoms may be due in some instances to potassium poisoning, while Macallum has obtained some data which suggest an increased potassium content of the serum in eclampsia. The data so far reported on pathological cases are too limited to permit any definite conclusions with regard to the findings. The observations of Myers and Short make improbable a definite potassium retention in chronic nephritis with marked nitrogen retention.

Calcium.—As has been shown by Abderhalden and others, the blood corpuscles are very low in their content of calcium. This being the case significant changes in the blood calcium are best shown, as pointed out

by Bergeim, by analyses made upon the serum or plasma. The serum normally contains 9 to 11 mg. of Ca per 100 c.c. in the healthy adult, also in infants. In advanced nephritis with acidosis and phosphate retention Marriott and Howland(*a*) have found the calcium of the serum to be markedly lowered, figures as low as 2 to 4 mg. More than ten years ago W. G. Macallum and Voegtlin recognized the reduction in the calcium content of the blood following the removal of the parathyroids in animals and the development of tetany. The symptoms of tetany were found to be relieved by the administration of calcium salts. Howland and Marriott, and more recently Denis and Talbot, have shown that the calcium content of the blood (serum) is greatly reduced in infantile tetany, falling to 2 to 3 mg. in some extreme instances. Howland and Marriott have shown that calcium administration produces a prompt effect upon the course of the tetany. In a few hours the spasmodic symptoms disappear. The calcium treatment must be continued, however, for a long time. Calcium chlorid administration causes an increase in the calcium of the serum coincident with the cessation of symptoms, although, in most instances, the calcium of the serum does not return to quite normal figures. Howland and Marriott point to the prompt improvement in infantile tetany after calcium medication and the absence of symptoms when the calcium of the blood remains above 7.5 mg. as strong evidence of the rôle that calcium plays in the production and dissipation of symptoms. Both Howland and Marriott, and Denis and Talbot have observed some decrease in the blood calcium in rickets, while Hess and Killian have noted a reduction in some cases of scurvy. It is a matter of clinical observation that in fractures occasionally cases are encountered which very rapidly regenerate bone, while others do so very slowly. It is natural to link this with deviations in calcium metabolism, but a few unpublished observations made in the writer's laboratory on patients of Drs. Albee and Moorhead have failed to disclose abnormal figures for the calcium of the serum.

Magnesium.—The normal magnesium content of the blood of both adults and children (as Mg generally falls between 2 and 3 mg. per 100 c.c. of plasma or serum, although with pathological bloods a somewhat wider range of 1 to 4 mg. is found. A considerable number of different pathological conditions have been studied, but the findings differ very little from those found during health and do not appear to be characteristic of any special pathological condition.

Iron.—As already pointed out, iron is present in hemoglobin to the extent of almost exactly one-third of one per cent, which would make the content of normal human blood about 50 mg. per 100 c.c. calculated as Fe. Pathologically, it varies directly with the hemoglobin content. Iron does not appear to be present normally in the plasma.

Chlorids.—Some of the observations recorded in the literature give the chlorid content of whole blood, others the content of the plasma or

serum. Normally the chlorid content of whole blood as NaCl amounts in round numbers to 0.45 to 0.50 per cent, while for the plasma the figures are about 0.12 per cent higher, i. e., 0.57 to 0.62 per cent. Since the plasma, rather than the whole blood, bathes the tissues of the body, it would seem more logical to study the chlorid content of the plasma. Unfortunately, unless the plasma is quickly separated from the corpuscles there appears to be a gradual change (increase) in its chlorid content, owing to a passage of carbon dioxid from the plasma into the corpuscles (or its escape into the air) and of chlorids from the corpuscles to the plasma. This being the case, results obtained on whole blood would appear to be more trustworthy than those obtained on plasma.

As far back as 1850 Carl Schmidt, in his classic studies on the blood with special reference to cholera, gave figures for the chlorid content of whole blood and plasma. Low figures were obtained in many cases of cholera, apparently as the result of the concentration of the blood, while in a case of "chronic edema with albuminuria" a definite increase was observed. McLean has devoted considerable attention to the subject of the chlorids of the blood working along lines similar to those of Ambard. In a fairly large series of normal individuals he found the plasma chlorid to vary from 0.57 to 0.62 per cent with a very constant chlorid threshold of about 0.562 per cent. The threshold was calculated from the formula of Ambard and Weill and confirms their observation on this point. McLean considered the question of the plasma chlorids in a number of pathological conditions, the lowest observation being 0.50 per cent in a diabetic and the highest 0.84 per cent in a cardionephritic shortly before death. In general, relatively increased concentrations of chlorids were found in the plasma in certain forms of cardiac and renal disease, while decreased concentrations were noted in certain diabetic and fever patients, also after the action of digitalis, the decreased concentrations apparently resulting from a temporary or permanent lowering of the chlorid threshold. Failure to excrete chlorids in pneumonia was found to be associated with a lowered concentration of chlorids in the plasma, excretion reappearing with a rise in the plasma chlorid. Edema was usually found to be accompanied by a relatively increased concentration of chlorids in the plasma, which ordinarily returned to the normal state with the disappearance of the edema.

In general it may be stated that high blood chlorids have been found in nephritis, certain cardiac conditions, anemia and some cases of malignancy (possibly due to an accompanying renal involvement), while low values have been observed notably in fevers, diabetes, pneumonia and Asiatic cholera. The chlorid retention in most cases of nephritis apparently results from impaired renal function. The excretion of chlorids and nitrogen seems to be a fairly independent renal function. In contrast to so-called parenchymatous nephritis, the function of excreting chlorids in

chronic (interstitial) nephritis appears to be much less impaired than excreting nitrogen. Consequently a restriction in the chlorid intake in the latter condition may fairly quickly restore the chlorids to normal. In fact, it is sometimes noted that when cases with marked nitrogen retention are put on a restricted chlorid diet, the blood chlorids fall to a subnormal level, such as is occasionally found in severe diabetes. A possible explanation for this is that, owing to the large amounts of urea and sugar present in the blood in these conditions, less chlorid is needed to maintain normal osmotic conditions. The high chlorid figures for whole blood in anemia and low figures in Asiatic cholera find probable explanation on the basis of the relatively high proportion of the plasma in the former disorder and the reverse condition in the latter.

Phosphates.—The presence of phosphorus in the blood in lipoid form has long been recognized, but exact data regarding the inorganic phosphorus is of more recent origin. In 1915 Greenwald (*c*) reported observations on the acid-soluble (largely inorganic) and lipoid phosphorus of human blood serum. He observed that normally the acid-soluble phosphorus as P varied between 2 and 6 mg. per 100 c.c., but that in severe nephritis it might be considerably increased. A year later Marriott and Howland (*a*) confirmed these observations and pointed out that the retention of (acid) phosphate would seem to be sufficient to account for the degree of acidosis observed. Recently Denis and Minot (*g*) have studied the inorganic phosphates of the plasma in a large series of pathological conditions. In conditions other than nephritis and cardiorenal disease figures varying from 1.2 to 3.1 mg. of P per 100 c.c. of plasma were found, while in one case of uremia figures exceeding 40 mg. were observed. They believe that the determination of the inorganic phosphate of the plasma gives promise of being of considerable prognostic value in renal and cardiorenal disease, since fatal cases which they examined showed a rapidly rising plasma phosphate.

An idea of the distribution of the various phosphorus compounds of normal human blood may be obtained from the table on page 454 taken from Bloor (*h*) (the figures have been recalculated to terms of P).

As is evident from the table below the phosphoric acid compounds of human blood may be divided into two classes: (1) the acid-soluble—soluble in dilute acids and precipitated with the proteins by alcohol-ether—and (2) the lipoid-phosphoric acid compounds—soluble in alcohol-ether and precipitated with the proteins by dilute acids. These two groups are apparently sharply defined and since their sum is practically equal to the total phosphates, the presence of other forms of phosphorus in blood in significant amounts is doubtful. Inorganic phosphates and an unknown compound which on decomposition by heating with acid yields phosphoric acid are present in the first group, while substances of the type of lecithin are found in the second group (lecithin has already been dis-

PHOSPHORUS CONTENT OF HUMAN BLOOD,
MILLIGRAMS P PER 100 C.C.

Sex	Plasma					Corpuscles				
	Total	Acid-Soluble	Inorganic	Lipoid	Other Forms	Total	Acid-Soluble	Inorganic	Lipoid	Other Forms
Men										
Low	7.6	2.3	1.9	5.0	0.1	57.8	43.8	3.8	13.6	40.0
High	13.6	4.3	3.7	7.3	1.2	101.5	78.1	8.5	20.8	74.2
Average (16 cases).	10.0	3.2	2.7	7.0	0.5	77.5	58.8	5.8	18.0	53.8
Women										
Low	9.9	2.9	2.5	6.0	0	68.1	50.0	3.0	14.7	41.8
High	12.6	4.5	4.3	9.1	1.2	82.8	64.4	8.2	19.5	58.8
Average (10 cases).	11.3	4.0	3.5	7.8	0.4	77.5	58.8	4.9	17.7	52.2

cussed, see p. 446). As will be noted the average content of inorganic phosphorus in the plasma of both men and women is about 3 mg. per 100 c.c. and of lipoid phosphorus about 7.5 mg. The corpuscles are relatively richer in all types of compounds than the plasma and there is also considerably less variation in their composition in different individuals than is the case with the plasma. The amount of the unknown form of phosphorus combination is very small, but in the corpuscles it constitutes 60 to 80 per cent of the total phosphorus. This large amount of organic phosphorus in the corpuscles is significant considering the fact that Bloor has shown that "lecithin" formation takes place in the corpuscles during fat absorption. Furthermore it would appear to be the mother substance of the phosphoric acid of the lipoid phosphorus compounds. Owing to the fact that this organic phosphorus compound is relatively unstable, it is probably easily made available to serve as a "buffer" in case of need.

Sulphates.—According to Greenwald(*d*) the sulphate sulphur of human blood plasma probably does not exceed 3 mg. per 100 c.c., although the content in the cells may be as high as 10 mg. The figures appear to be considerably increased in some cases of nephritis.

Blood Gases

Although we possessed considerable information regarding the blood gases as a result of observations made with the Barcroft-Haldane method, the development by Van Slyke(*c*) of a much simpler method of estimating the oxygen and carbon dioxid of the blood has given a considerable impetus to this line of study. For the extraction of the gas to be determined, Van Slyke makes use of a Torricellian vacuum, with which the gas is easily and completely extracted in a closed chamber without any loss. Furthermore, the Haldane apparatus has recently been considerably simplified by Hen-

derson, and application made to the blood gases by Henderson and Smith. Very recently Van Slyke and Stadie have introduced a number of different refinements in the Van Slyke method of gas analysis and it would seem that this method now left little to be desired in the point of accuracy.

The great practical importance of a knowledge of the factors concerned in the carrying of oxygen to the tissues and the removal of carbon dioxid is apparent.

Oxygen.—As has already been pointed out, the ability of the blood to absorb and take up oxygen depends upon its hemoglobin content. Since hemoglobin so readily takes up and gives off oxygen, it is obvious that venous blood should be partly unsaturated and therefore differ from the arterial blood in respect to its oxygen content, and further that blood obtained from different parts of the venous system should differ in its oxygen unsaturation. Extensive studies on the venous blood from single organs have been made in animals by Barcroft and his associates, but in the human adult the superficial veins of the limbs and neck, particularly of the arm (vena mediana), are the only sources from which venous blood can be obtained. This means that in the human only blood coming from a limited region, consisting chiefly of muscles, can be studied.

Lunsgaard(*a*) has given the following figures for the oxygen content and oxygen unsaturation of the venous blood of the normal resting adult. The results are the average of thirty-eight determinations on twelve individuals and are given in tabular form below:

Oxygen Content of Venous Blood Volume Per Cent			Oxygen Unsaturation of Venous Blood Volume Per Cent		
Maximum	Minimum	Average	Maximum	Minimum	Average
18.0	9.6	13.6	9.0	2.7	5.8

In studying this question on circulatory disorders, Lunsgaard(*b*) found that in twelve patients with compensated heart lesions the unsaturation fell within normal limits, between 2.5 and 8 volume per cent, while in four patients with uncompensated heart disease the values for the unsaturation were all above the normal limits, from 9.7 to 15.2 volume per cent. In these cases the oxygen unsaturation appears to afford an objective criterion of the positive effect of digitalis therapy. From studies performed on patients with varying amounts of hemoglobin it has been shown that the oxygen unsaturation of the venous blood is independent of the oxygen capacity, unless the latter is reduced below the normal value for oxygen unsaturation (about 5 volumes per cent). Lunsgaard found, for example, that in a polycythemic patient with an oxygen capacity of 33.4 volumes per cent, the venous oxygen unsaturation was 5.4 volumes per cent, while

in an anemic patient with an oxygen capacity of 6.7 volumes per cent the venous oxygen unsaturation was 5.2 volumes per cent, indicating that the tissues extract from the blood all the oxygen they need with apparently equal readiness, regardless of whether the extraction leaves a great oxygen reserve in the blood as in polycythemia, or practically no reserve as in anemia.

Considerable additional information may also be obtained when the study of the oxygen content of the arterial blood is included. Such studies have been conducted on normal and certain pathological conditions by Stadie and by Harrop(*b*), the arterial blood being obtained from the radial artery. Observations obtained by Stadie for the arterial and venous oxygen, and total oxygen capacity of five normal resting men are given in the table below. As will be noted the arterial unsaturation amounts to

Individual	Oxygen Content		Oxygen Capacity per 100 c.c. of Blood	Unsaturation			
				Arterial		Venous	
	Arterial per 100 c.c. of Blood	Venous per 100 c.c. of Blood		Per 100 c.c. of Blood	Per Cent	Per 100 c.c. of Blood	Per Cent
	c.c.	c.c.	c.c.	c.c.		c.c.	
1	17.9	12.8	19.1	1.2	6.3	6.3	33.0
2	21.0	16.7	21.6	0.6	2.8	4.9	22.7
3	22.1	17.2	23.3	1.2	5.2	6.1	26.2
4	20.2	15.6	21.6	1.4	6.5	6.0	27.8
5	19.5	15.4	20.3	0.8	3.9	4.9	24.1
Mean	20.2	15.6	21.2	1.0	5.0	5.6	26.8

about 5 per cent while the venous unsaturation slightly exceeds 25 per cent. Similar studies were made on a series of pneumonia cases (chiefly post influenza), a high arterial unsaturation being observed in the fatal cases. A definite relation was found to exist between the degree of cyanosis and the per cent of arterial unsaturation. With increasing cyanosis the arterial unsaturation becomes greater. The venous saturation varies similarly. Obviously the cyanosis of pneumonia patients is due to the incomplete saturation of venous blood with oxygen in the lungs. The range of arterial and venous unsaturation encountered in fatal and nonfatal cases of pneumonia is well illustrated in the table below, taken from Stadie. As will be noted the arterial unsaturation of the fatal cases aver-

Type of Cases	No. of Cases	Arterial Unsaturation			Venous Unsaturation		
		Max.	Min.	Mean	Max.	Min.	Mean
Normal individuals	5	6.5	2.8	5.0	33.0	22.7	26.8
Nonfatal cases ...	16	33.0	1.6	13.9	61.2	14.4	36.3
Fatal cases	16	68.2	14.1	32.0	85.5	22.3	57.0

aged 32 per cent and in one case reached 68 per cent, the venous unsaturation exceeding 85 per cent.

The oxygen content of the arterial blood in anemia and heart disease has been studied by Harrop, who likewise made a careful study of the blood gases (oxygen and carbon dioxid) in both the arterial and venous blood of fifteen normal subjects, his figures for oxygen agreeing closely with those of Stadie. With severe anemia the saturation of the arterial blood did not differ from the normal. Low absolute values were found for the oxygen content of the venous blood, but the normal oxygen consumption was maintained. No deviations from the normal were found in arterial and venous blood from cardiac patients without arrhythmias, well compensated, and at rest in bed. With cardiac cases showing varying degrees of decompensation the arterial unsaturation is frequently abnormally low (sometimes exceeding 15 per cent), although not so low as that found in pneumonia. It is apparent that in many circulatory diseases during decompensation, particularly when there are physical signs of pulmonary congestion, there is a disturbance of the pulmonary exchange, as indicated by the lowering of the percentage saturation of the arterial blood with oxygen.

Carbon Dioxid.—Recent studies on the carbon dioxid of the blood have been devoted largely to the utilization of this determination as a means of ascertaining the carbon dioxid capacity of the blood. This determination, as Van Slyke and Cullen have pointed out, furnishes a most excellent method of ascertaining the degree of an acidosis, since the bicarbonate of the blood represents the excess of base which is left after all non-volatile acids have been neutralized and in this sense constitutes the *alkaline reserve* of the body. Before entering into a discussion of this phase of the subject, however, it may be well to consider the actual content of carbon dioxid in normal human blood.

Harrop(*b*) has presented some interesting figures for the oxygen and carbon dioxid content (according to the Van Slyke method) of both arterial and venous blood upon individuals with normal heart and lung findings. A few of these are given in the table on page 458.

As will be noted the CO_2 content of arterial blood in the first six cases tabulated averages about 50 volumes per cent, while that of the venous blood is 4 volumes per cent higher. After 15 minutes of brisk exercise Harrop found the CO_2 content of both arterial and venous blood reduced, with a considerable increase in the venous-arterial whole blood difference. The oxygen consumption was, however, only slightly increased.

Smith, Means and Woodwell, employing the Henderson apparatus, found the CO_2 content of eight normal whole bloods to average 50.4 volumes per cent, while the venous blood showed 58.7 volumes per cent, a difference of 8.3, which is considerably greater than that recorded below.

Individual	Oxygen Capacity	Oxygen Content of Arterial Blood	Percentage Arterial Saturation	Arterial Oxygen Unsaturation	Oxygen Content of Venous Blood	Oxygen Consumption	CO_2 Content of Arterial Blood	CO_2 Content of Venous Blood
1	23.7	23.0	98	0.7	17.6	6.4	51.8	57.2
2	17.2	17.2	100	0.0	14.6	2.6	54.7	56.7
3	16.3	15.3	94	1.0	10.5	4.8	52.9	55.9
4	20.6	19.8	96	0.8	13.5	6.3	46.5	51.7
5	18.7	17.8	95	0.9	15.1	2.7	44.8	48.3
6	20.6	19.8	96	0.8	12.7	7.1	49.7	54.6
Average	19.5	18.8	96	0.7	14.0	5.0	50.1	54.1
Normal adult, resting	22.0	21.1	96	0.9	15.1	6.0	53.3	56.9
After exercise	22.4	19.2	86	3.2	12.9	6.3	32.3	41.1

According to these workers, as the blood passes from the arterial to the venous side of the circulation in normal man its cells gain from 4 to 11 volumes per cent of CO_2, while the corresponding gain in the plasma is only from 0 to 1.8 volumes per cent, indicating that the transport is accomplished mainly by the cells. Theories regarding the ability of the blood to take up and hold oxygen and carbon dioxid and the equilibrium between these two gases in the blood have recently been presented by L. J. Henderson(*c*) and Y. Henderson and Haggard(*b*).

Although the removal of carbon dioxid from the tissues may be accomplished mainly through the agency of the cells, still the bicarbonate of the plasma is ordinarily in equilibrium with that of the cells, as Van Slyke and Cullen have pointed out. Consequently the carbon dioxid capacity of the plasma may be used as a simple practical method of measuring the alkaline reserve of the body. (Whole blood may be used, and theoretically is to be preferred, but it easily gums up the Van Slyke apparatus.)

Acidosis may result from an abnormal formation of acid substances such as is found in diabetes, or from a decreased elimination of normally formed substances as in nephritis. The carbonates of the blood have been called by L. J. Henderson the first line of defense against acidosis. Increased pulmonary ventilation as occurs with dyspnea or hyperpnea, serves to increase the excretion of carbon dioxid, thus keeping the reaction of the blood within normal limits. In conditions of acidosis, other acids may combine with the bicarbonate, robbing the body of its alkaline reserve. In diabetes this is brought about by the abnormal formation of ketone bodies, while in nephritis the breakdown in the excretion of acid phosphate apparently brings about the same result.

The range of the carbon dioxid combining power of the blood plasma of the normal resting adult, with the Van Slyke(*e*) method, is from 55 to 75 c.c. of CO_2 per 100 c.c., with an average of 65 c.c. For normal in-

fants the figures are about 10 c.c. lower than in adults. With moderate acidosis, in which symptoms may or may not be apparent, CO_2 combining power figures of 30 or below are found. In the terminal stages of diabetic coma figures of 10 to 15 c.c. are encountered, and similar figures are sometimes observed in "uremia." In such cases death may be directly ascribed to the acidosis. Extremely low figures are encountered in many cases dying from pneumonia. Low figures may likewise be obtained in the diarrheal acidoses of infancy. All cases of chronic nephritis with marked nitrogen retention show a moderately severe or a severe acidosis, while occasionally severe acidosis is encountered in acute nephritis. Ether anesthesia is accompanied by a fall in the CO_2 combining power of the blood, amounting to 2 to 20 volumes per cent. The introduction of a simple method of estimating the CO_2 combining power of the blood has placed the diagnosis and treatment of cases of acidosis on a rational basis.

Muscle

The muscle tissue of the human adult has been variously estimated as comprising from 30 to 40 per cent of the body weight. Of the total body metabolism about 50 per cent takes place in the muscles during rest and 75 per cent during activity. Physiologically, muscle tissues are divided into voluntary or striated and involuntary or non-striated muscle, heart muscle belonging to an intermediate group. The involuntary muscles comprise only a comparatively small part of the total muscle tissue. The muscle fibers of which muscle tissue is chiefly composed are elongated, spindle shaped cells. Muscle tissue in the adult contains from 22 to 28 per cent solids with an average of 25 per cent. Of this about four-fifths is protein and the remainder largely extractives and inorganic salts.

The proteins of the muscle are ordinarily divided into two groups, the muscle plasma and the muscle stroma. This division or separation is a more or less arbitrary one, since the muscle plasma simply represents the amount of protein which can be expressed (about 60 per cent) from fresh muscle. In the muscle plasma there are two distinct proteins, as may readily be shown by the fractional coagulation of the plasma. Paramyosinogen (Halliburton(a)) or myosin (von Fürth(a)) coagulates at 46-51°C., while myosinogen or myogen coagulates at 55-65° C. The former constitutes about 25 per cent of the protein in the plasma and the latter 75 per cent. The first of these proteins is definitely a globulin, but the latter is not a typical globulin since it is soluble in water, and belongs rather to the class of albumins. The proteins of different muscles do not differ widely in their content of amino-acids. The phenomenon of *rigor mortis,* according to the now generally accepted view, first suggested by Meigs, is due to the swelling of the muscle cells (taking up of water)

as a result of the post-mortem formation of lactic acid, increasing the hydrophylic properties of the protein colloids of the muscle.

The so-called extractives of muscle are of considerable interest and importance. Including the inorganic salts they constitute about 2 per cent of the tissue, the organic material amounting to 0.7 per cent and the inorganic to 1.3 per cent. The organic material is ordinarily divided into two groups, the non-nitrogenous and the nitrogenous. To the former group belong glycogen, glucose, para- or sarcolactic acid and inositol, and to the latter such substances as creatin, the purin bases, xanthin, hypoxanthin and guanin, carnosin, amino-acids and traces of creatinin, uric acid and urea.

Glycogen is a polysaccharide carbohydrate possessing some of the properties of starch and dextrin. It is present in normal human muscle tissue to the extent of about 0.5 per cent. From experiments on animals we know that the amount may be markedly reduced by muscular activity. Glycogen constitutes the muscles' reserve supply of energy. The glycogen of the muscle together with that of the liver is apparently transformed to glucose as needed. Judging from the observations of Palmer the glucose content of the muscle is only about half that of the blood. Hopkins has recently presented some interesting views regarding the transformation of glycogen into mechanical and heat energy. The facts which he has brought together indicate that there are two phases in muscular activity, the first anaërobic and the latter aërobic. During the first, in which muscular contraction takes place, lactic acid is formed. During the second phase a part of the lactic acid is oxidized and transformed to carbon dioxid and water, while a part is apparently reconverted to glycogen. The heat liberated during this (second) period, however, is less than that required by the oxidation of the lactic acid, and is apparently stored in the muscle in a latent form for the next (first) phase of the reaction, when it is liberated. The formation of lactic acid (producing changes in the hydrogen ion of the muscle) apparently plays an important rôle in initiating the contraction of the muscle, while a combination of the glucose with phosphoric acid is necessary to its cleavage into lactic acid. Rigor may take place in the muscle as a result of severe exertion or from poor oxidation as in carbon monoxid poisoning, while rigor mortis may be prevented if a sufficiently high concentration of oxygen is maintained to bring about an oxidation of lactic acid. However, after a time irritability is lost apparently as a result of the stabilization of the inorganic ions by the tissue. Although inositol possesses the same empirical formula as glucose, it is a hexahydroxybenzene. However, it probably stands in fairly close relationship to sugar since lactic acid may be formed from it.

Of the nitrogenous extractives of muscle, creatin is present in much the largest amount and is of the greatest interest, especially since it is apparently the precursor of the creatinin of the urine. In 1913 Myers and

Fine called attention to the fact that the creatin content of the muscle of a given species of animals was very constant (obviously that of a given animal) and suggested this as a possible basis of the constancy in the daily elimination of creatinin first noted by Folin. Later they pointed out that the creatinin content of muscle was greater than that of any other tissue, and also that in autolysis experiments with muscle tissue the creatin (and any added creatin) was converted to creatinin at a constant rate of about 2 per cent daily, which is just about the normal ratio between the muscle creatin and urinary creatinin. They also found that, when creatin was administered to man or animals, there was a slight conversion to creatinin which corresponds well with the above figure. These facts all go to support the view that creatinin is formed in the muscle tissue from creatin, and at a very constant rate, although no explanation of the physiological significance of this transformation can as yet be offered.

For the rabbit Myers and Fine(*c*) found a creatin content of 0.52 per cent, for the white rat 0.47 per cent, for the dog 0.37 per cent and for two human cases 0.39 per cent. This figure for normal human muscle was likewise confirmed by Denis(*e*) who has reported data for the muscle creatin on nearly a hundred human cases. In a series of determinations made on persons dying from various chronic diseases the creatin of the muscle was found to be reduced absolutely and relatively in many cases, especially those in an emaciated condition. These are the type of cases which excrete creatin and show low creatinin coefficients. Denis likewise found the percentage of muscle creatin in children to be lower than that of adults, which is in harmony with the observation that children excrete creatin.

Of the nitrogenous extractives carnosin stands next to creatin in point of quantity. It is a dipeptid containing histidin and alanin. By its synthesis Baumann and Ingvaldsen(*b*) have shown carnosin to be β-alanylhistidin. Figures given for its contents in muscle vary from 0.035 to 0.30 per cent. About 0.05 per cent has been reported for human muscle.

The amount of purin base nitrogen found in the muscle of mammals is generally given as about 0.05 per cent. This is partly combined and partly free. From the observations of Davis and Benedict on a combined uric acid compound present in beef blood, it is apparent that purins may even be oxidized to uric acid before they are split off from the sugar with which they are combined in the nucleic acid molecule. Of the different purins hypoxanthin is generally stated to be present in the largest amount, although both xanthin and guanin are also present.

As was pointed out by Marshall and Davis urea is so diffusible that it is very evenly distributed throughout the tissues of the body, and this has been amply confirmed by the observations of Mosenthal, Clausen and Hiller on human muscle tissue in cases with and without nitrogen retention.

Normally muscle tissue contains rather more creatinin than the blood, but in cases of marked nitrogen retention the blood may slightly exceed that of the muscle (Myers and Fine (*c*)). The uric acid of the muscle scarcely keeps pace with the rise in the blood uric acid which occurs in some cases of advanced nephritis. The figures for the non-protein nitrogen of muscle are much higher than those of the blood, owing chiefly to the much larger amounts of creatin and amino-acid nitrogen present in muscle than in blood.

The table below compiled from observations of Mosenthal, Clausen and Hiller, and Myers and Fine (b) gives an idea of the distribution of the various non-protein nitrogenous constituents in the muscle tissue of normal individuals and those suffering from severe nephritis.

Content of Nitrogeneous Constituents in Human Muscle

Determination	Normal	Severe Nephritis
Total solids ..... per cent	24	...
Total nitrogen ..... " "	3.5	...
Total nonprotein N ..... mg. to 100 gms.	185	375
Creatin N ..... " " " "	125	125
Amino-acid N ..... " " " "	35	30
Urea N ..... " " " "	13	200
Creatinin N ..... " " " "	1	5
Uric acid N ..... " " " "	0.5	2

It is very difficult to completely free muscle tissue from adherent fat. Figures as low as 0.6 per cent have been obtained in lean oxen and as high as 9 per cent in fattened pigs. Less is known concerning the cholesterol and phosphatids of the muscle, although the latter are present in much higher concentration, especially in heart muscle.

One may obtain an idea of the inorganic constituents of muscle from the following table taken from Katz(*b*). Of the different constituents tabulated potassium and phosphorus are present in by far the largest amounts.

Mineral Content of the Muscle of Mammals

Constituent	Range in Mammals	Man
	Per Cent	Per Cent
Potassium	0.254–0.398	0.320
Sodium	0.065–0.156	0.080
Iron	0.004–0.024	0.015
Calcium	0.002–0.018	0.021
Magnesium	0.021–0.030	.....
Phosphorus	0.170–0.253	0.203
Chlorin	0.040–0.081	0.070
Sulphur	0.186–0.227	0.208

In striated muscle the phosphorus is present largely in inorganic form, but in heart muscle organic phosphorus may constitute more than half of the phosphorus present. In the voluntary muscle of the rabbit, which

has a relatively high content of creatin, Myers has observed that the potassium is present in fairly high concentration, 0.46 per cent calculated as K (average for 8 animals). In conditions such as starvation, which ultimately bring about a reduction in the creatin, it is of interest that the potassium, as a rule, shows a proportionate reduction.

Without further discussion it may be said that there are many observations which lead one to believe that glycogen, creatin, phosphoric acid and potassium are closely associated in active muscle.

Liver and the Bile

An appreciation of the importance of the liver to the animal organism may be gained from the following facts. The liver is the largest gland of the body. Its extirpation in mammals quickly results in death. The blood from the digestive tract first passes through the liver before reaching the general circulation. The liver appears to be a temporary storehouse for all classes of foodstuffs, carbohydrate (glycogen), fat and protein (amino-acids). Many poisons both inorganic and organic are retained by the liver, many of the latter being detoxicated. Numerous chemical reactions, in which deamidization, hydrolysis, oxidation and reduction occur, take place in the liver. The liver also appears to be chiefly concerned in the synthesis of urea (uric acid in birds), sugar from protein and the ethereal sulphates. The formation of fibrinogen and also serum albumin and globulin has been ascribed to the liver.

Less is known concerning the proteins of the liver than of the muscle. There are two proteins, apparently globulins, which coagulate at 45° and 75° respectively, and a nucleoprotein which coagulates at 70° C. Besides these proteins which are soluble there are others in the cells which are difficultly soluble. The fat (fatty infiltration) of the liver is derived not only from an excess of fat in the diet, but also by transportation from other parts of the body. The phosphatids (lecithin) are normal constituents of the liver and are subject to much less variation than the fat. Cholesterol is also a normal constituent but found in small amounts. As in the muscle, phosphoric acid and potassium are the mineral constituents which are present in the highest concentrations. Compared to other tissues iron appears to be present in fairly large amounts. It is of interest that considerable iron is stored in the liver during fetal life, apparently to provide for the deficiency in the diet during the period of lactation.

The storing of carbohydrate in the liver in the form of glycogen is one of the liver's many important functions. The credit for the discovery of glycogen and this glycogenic function of the liver, i. e., the ability of the liver to convert glucose to glycogen and glycogen to glucose, is due to Bernard. In normal animals the quantity of glycogen in

the liver depends essentially upon the food intake. In starvation the glycogen may almost disappear from the liver, but after food very rich in carbohydrate it may in exceptional cases reach nearly 20 per cent. Apparently only the fermentible sugars of the six carbon series or their di- and polysaccharids are true glycogenformers. The di- and polysaccharids must, however, first be broken down into monosaccharids in digestion. Glucose is apparently more readily converted into glycogen than fructose, and much more readily than galactose. These transformations are apparently brought about by the diastatic ferment of the liver. The liver is the probable source of the blood diastase. It is of interest that in diabetes, where the reserve supply of glycogen in the liver is very small, the diastatic activity of the blood is generally markedly increased. It is further significant that when the liver is cut out of the circulation in animals, the blood sugar rapidly falls and may almost disappear. The influence of the various internal secretions and also Bernard's sugar puncture are of considerable interest and importance in this connection. As regards the formation of sugar from protein it would seem probable that the liver was chiefly concerned in the deamidization of amino-acids and the transformation of the carbon moiety to sugar. Not all amino-acids are sugar-formers, although it may be noted that practically all the amino-acids with straight chains, except lysin, yield sugar. Prolin is the only cyclic amino-acid which produces an abundance of sugar.

That urea formation takes place in the liver is unquestioned as a result of the well-known experiments of von Schroeder and others. That the liver is the only organ in the body where urea formation takes place seems improbable, still the actual demonstration of the formation of urea elsewhere than in the liver has not been made. In autolysis experiments M. Ringer was able to demonstrate urea formation in liver tissue but not in muscle tissue. Muscle tissue added to liver tissue was found, however, to augment the urea formation. It would appear that the liver was the chief organ concerned in the synthesis of urea, apparently deamidizing the amino-acids no longer of use to the body or in excess of the body's requirements. In the case of the amino-acid, arginin, Kossel and Dakin have shown that a specific liver enzyme, arginase, converts the arginin to ornithin and urea.

The liver has its own secretion, bile, which it continuously secretes; a reservoir, the gall bladder, being provided, so that the bile need not be discharged into the intestine except as required. The discharge of bile is brought about by the same stimulus that initiates the secretion of pancreatic juice, namely secretin. Bile may be regarded not only as a secretion but also as an excretion, since it carries to the intestine certain metals, cholesterol, lecithin, decomposition products of hemoglobin, and certain foreign organic substances, for example, tetrachlorphthalein.

In man bile is usually a golden yellow, rather viscid fluid, amounting

to roughly 500 to 1000 c.c. in 24 hrs. It is usually alkaline in reaction to litmus, and ordinarily possesses a decidedly bitter taste. The specific gravity varies between 1.010 and 1.040. As secreted by the liver bile is a rather limpid fluid, but the addition of mucus and the abstraction of water in the gall bladder raise both the specific gravity and the viscosity. The table below, compiled from analyses given by Hammarsten, gives a good idea of bladder and liver bile.

Constituents	Liver Bile (Hammarsten)			Bladder Bile (Frerichs)	
	I	II	III	I	II
	Per Cent	Per Cent	Per Cent		
Water	97.48	96.47	97.46	86.0	85.9
Solids	2.52	3.53	2.54	14.0	14.1
Mucin and pigments	0.53	0.43	0.52	2.7	3.0
Bile salts	0.93	1.82	0.90	7.2	9.1
Taurocholate	0.30	0.20	0.22	....	....
Glycocholate	0.63	1.62	0.68	....	....
Fatty acids and soaps	0.12	0.14	0.10	....	....
Cholesterol	0.06	0.16	0.15	0.16	0.26
Lecithin	0.02	0.06	0.07	....	....
Fat		0.10	0.06	0.32	0.92
Soluble salts	0.81	0.68	0.73	0.65	0.77
Insoluble salts	0.25	0.05	0.02		

The most important constituents of bile are the bile acids and bile pigments. The bile acids may be divided into two groups, the glycocholic and taurocholic acid groups, the former being considerably in excess in human bile as indicated in the table above. The bile acids are conjugate amino-acids, in which glycocoll or taurin are joined to cholic acid. This latter acid exists in several forms. There is some reason for believing that cholic acid is derived from cholesterol. The bile acids generally exist in the bile in the form of sodium salts. The bile salts have the power of holding the cholesterol and lecithin of the bile in solution. They also act as a coferment to the pancreatic lipase, thus facilitating fat digestion. The bile salts have a strong hemolytic action on the red blood cells.

The bile pigments are derived from the decomposition of the hematin portion of hemoglobin, after the removal of the iron. (Whipple and Hooper(*b*) have recently suggested the possibility of another origin.) Although the liver is apparently chiefly concerned in this transformation, the formation of the bile pigments may take place elsewhere in the body. Bilirubin and biliverdin, an oxidation product of bilirubin, are the two chief bile pigments, the one possessing a golden yellow and the other an emerald green color. Bilirubin is identical with the hematoidin of old blood clots, and isomeric with the hematoporphyrin of pathological urines. Under the action of intestinal bacteria bilirubin is reduced. It would appear that hydrobilirubin prepared by the chemical reduction of bilirubin, the stercobilin of the feces and the urobilin of the urine were

practically the same substance. It has become customary to refer to the pigment of both feces and urine as urobilin. Urobilin is generally excreted to a large extent in the form of a chromogen, urobilinogen, which on exposure to light is converted to urobilin. Normally a considerable part of the urobilin(ogen) of the intestines is reabsorbed and reconverted to bile pigments. In certain diseases of the liver, the liver cells partially lose this capacity, thus giving rise to an increased excretion of urobilinogen in the urine. Owing to the greatly increased destruction of red cells in pernicious anemia (but not in secondary anemia) the output of urobilin in the stool is greatly increased, an observation which is of considerable value in differentiating the two forms of anemia.

Human biliary calculi or gallstones are as a rule composed largely of cholesterol in man. Occasionally the stones are pearly white, indicating that they are almost entirely cholesterol, although more often they are somewhat pigmented, sometimes very much so, indicating a mixture with calcium salts of bilirubin and biliverdin. Stones made up largely of pigments are not often found in man. The etiology of gallstone formation is not as yet clear.

Connective Tissues

The cellular elements of typical connective tissues and gelatin-yielding fibrils are imbedded in an interstitial or intracellular substance. The fibrils consist of *collagen,* while the interstitial substance contains chiefly mucoid, besides small amounts of albumin and globulin. In yellow elastic tissue, fibrils containing elastin are also present. Four types of connective tissue will be mentioned, (1) white fibrous tissue, (2) yellow elastic tissue, (3) cartilage and (4) bone.

The tendo Achillis is generally taken as a typical example of white fibrous tissue. According to the analyses of Buerger and Gies, the tendo Achillis of the ox contains 31.6 per cent of *collagen* in the fresh tissue and 85 per cent in the dry tissue, together with 4.4 per cent of elastin and 3.5 per cent of mucoid.

The ligamentum nuchæ of the ox is the classic illustration of yellow elastic tissue. Vandegrift and Gies give the content of *elastin* in the fresh tissue as 31.7 per cent, and in the dry tissue as 74.6 per cent, together with 17 per cent of collagen and 1.2 per cent of mucoid.

Cartilage is closely related to white connective tissue, since it contains a relatively large amount of collagen. In addition it contains an albuminoid, chondroalbuminoid, and chondroitin-sulphuric acid. Chondromucoid differs from the mucoids found in other connective tissues in the large amount of chondroitin-sulphuric acid obtained on decomposition. This acid is also found in bone, ligament and other tissues. Under the action of acid hydrolysis, *chondroitin* is first formed, then later *chondrosin.*

Chondrosin has a very strong reducing action, which is due to a hexosamine, named by Levene and La Forge *chondrosamine,* since it is isomeric but not identical with glucosamine. Levene(*c*) has recently shown that it is a derivative of galactose. *Glucuronic acid* is also present in the molecule of chondroitin-sulphuric acid.

The organic intracellular substance of bone is very similar to cartilage. It differs in its very large deposit of *inorganic salts,* which normally constitute about 40 per cent of the dry weight of the tissue. The *ossein* of bone differs in no essential from the collagen of the other tissues mentioned. Likewise the osseomucoid and osseo-albuminoid are similar to those found in tendon and cartilage. The inorganic material of bone is chiefly calcium phosphate and carbonate, but magnesium is present and also traces of fluorid and chlorid. McCrudden has given the following figures for the important inorganic constituents of normal human bone and bone from a case of osteomalacia:

Constituents	Normal	Osteomalacia
	Per Cent	Per Cent
Calcium as CaO	28.85	15.44
Magnesium as MgO	0.14	0.57
Phosphorus as P_2O_5	19.55	12.01
Sulphur as S	0.14	0.55

Brain

The adult human brain weighs about 1200 to 2000 grams, of which approximately 19 per cent is water. It contains from 100 to 120 grams of protein after the extraction of the various lipoids. The brain as a tissue is characterized by its very high content of lipoids, i.e., alcohol and ether soluble material. The first worker to make real progress in the chemistry of the brain was Thudichum, who published a most important monograph on the subject in 1884. Of more recent work the studies of Waldemar Koch(*a*) deserve special mention, while very important contributions regarding the constitution of many of the lipoid compounds of brain tissue have recently been made by Levene and his coworkers.

Among the solid constituents of brain tissue are proteins, phosphatids (lecithin, cephalin, etc.), cerebrosids or galactosids (phrenosin and cerasin), cholesterol, collagen, extractives and inorganic salts. Three distinct proteins, two globulins and a nucleoprotein, have been isolated from the brain. The globulins coagulate at 47° C. and at 70-75° C., while the nucleoprotein coagulates at 56-60° C. The lipoids are of particular interest and will be specially considered. These bodies, as their name would imply, resemble fats in some of their physical properties and

reactions, but are distinct chemically. The content of lipoids in the white matter of the brain is very much higher than in the gray matter. A general idea of the distribution of these various substances in human brain tissue may be obtained from the table below taken from Koch. It will be observed that the brain of the adult differs very materially from the child, notably in its higher content of lipoids, particularly cholesterol. With this increase in lipoids there is a corresponding reduction in protein, extractives and ash.

Composition of the Solids of the Human Brain

Constituents	In Per Cent of Dry Matter		
	Whole Brain (Child)	Whole Brain (Adult)	Corpus Callosum
Protein	46.6	37.1	27.1
Extractives	12.0	6.7	3.9
Ash	8.3	4.2	2.4
Phosphatids	24.2	27.3	31.0
Cerebrosids	6.9	13.6	18.0
Lipoid sulphur	0.1	0.3	0.5
Cholesterol	1.8	10.9	17.1

Possibly a better notion of the changes in the composition of the brain during growth may be obtained from data given by W. and M. L. Koch on white rats at different age periods. As will be observed well-marked and characteristic chemical changes occur in the rat during its growth which may be correlated with its anatomical differentiation. The principal changes are: "(1) A general decrease in the per cent of the water which is not due entirely to medullation, since the decrease begins before medullation; (2) a diminution in the relative per cent of protein in the total solids due to the formation of a large amount of lipoid matter; (3) the lipoids which appear coincident with medullation and of which the development is *pari passu* with medullation are the cerebrosids and phosphatids. These, therefore, are chiefly found in the medullary sheaths. (4) There is a great outburst of phosphatid formation at the very beginning of medullation. The phosphatids are present, therefore, in the cells as well as the sheaths."

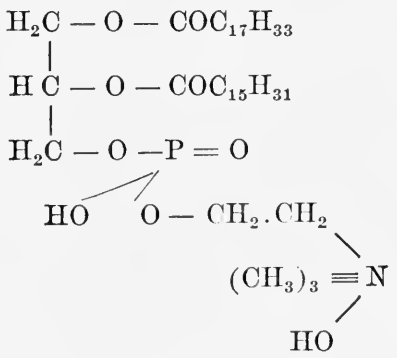
The chemistry, so far as known, of the various lipoid substances present in brain is of considerable interest. From the studies of Posner and Gies, and others, it is apparent that the nitrogenous phosphorized substance isolated by Liebreich and named "protagon" is a mixture.

Phosphatids.—The best examples of the phosphatids are lecithin and cephalin. Recently Levene and West have shown that it is possible to prepare perfectly pure *lecithin*. The lecithin molecule is known to be made up of two molecules of fatty acid, one of glycerol, one of phosphoric acid and one of the base, cholin. The lecithin of brain tissue appears to

THE RELATIVE PROPORTIONS OF THE CONSTITUENTS OF THE BRAIN OF THE ALBINO RAT AT DIFFERENT AGES

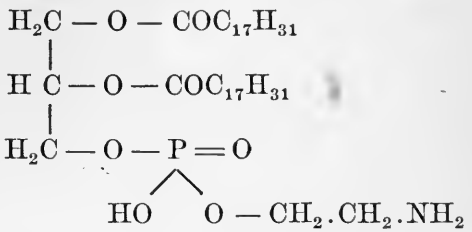
	Age in Days					
	1	10	20	40	120	210
Solids in per cent.....	10.42	12.5	17.5	20.34	21.65	21.9
Number of brains in each sample	100	40	54	35	30	31
CONSTITUENTS IN PER CENT OF TOTAL SOLIDS						
Proteins	58.25	56.5	53.3	48.4	47.6	48.5
Phosphatids	15.2	12.3	21.4	21.8	21.6	22.0
Cerebrosids			3.0	5.9	8.4	8.4
Sulphatids	1.45	2.6	2.5	2.55	3.55	4.5
Organic extractives ..... } Inorganic extractives .... }	17.9	15.1	14.55	14.85	9.75	9.8
Cholesterol (by difference).	7.2	13.5	5.25	6.5	9.1	6.8
Total sulphur	1.00	0.83	0.70	0.55	0.56	0.58
Total phosphorus	1.87	1.48	1.66	1.52	1.42	1.39
DISTRIBUTION OF SULPHUR IN PER CENT OF TOTAL S						
Protein S.....	30.5	44.2	56.4	63.75	61.8	63.8
Lipoid S.....	3.0	6.1	7.1	9.65	12.7	15.6
Neutral S.....	48.2	45.4	28.6	18.15	18.7	14.5
Inorganic S.....	18.3	4.3	7.9	8.45	6.8	6.1
DISTRIBUTION OF PHOSPHORUS IN TERMS OF TOTAL P						
Protein P.....	13.3	13.45	5.9	8.7	7.3	6.8
Lipoid P.....	33.2	34.95	52.85	57.3	64.1	67.6
Water Soluble P.....	53.5	51.6	41.25	34.0	28.6	25.6

contain one molecule of oleic and one of palmitic acid as the fatty acids. The formula would thus be written:



Cephalin differs from lecithin chiefly in containing as its basic substance amino-ethyl alcohol instead of cholin. Levene and Rolf have shown

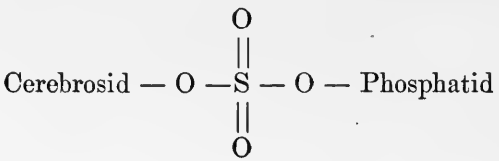
that the glycerophosphoric acid of cephalin is identical with that of lecithin. It also appears to contain another unsaturated fatty acid, namely, cephalinic acid, in place of oleic acid. The formula would thus be:



Two other monaminophosphatids found in brain tissue are *paramyelin* and *myelin,* the latter being present only in very small amounts. Diaminomonophosphatids are also present in brain tissue. Two have been recognized, *amidomyelin* and *sphingomyelin.* In the case of this latter compound Thudichum recognized that it did not contain glycerol. Levene has recently obtained on hydrolyzing sphingomyelin, phosphoric acid, two fatty acids, cerebronic and lingoceric, and three basic substances, cholin, sphingosin and a base of the composition $C_{17}H_{35}NO$.

Cerebrosids.—The cerebrosids are nitrogenous substances free from phosphorus, which yield galactose on boiling with dilute mineral acids. They also contain a complex fatty acid. As would seem evident from the table above they are not found in the embryonic brain, but develop as medullation comes on and are found chiefly in the medullary sheaths in the white matter of the brain. The most important of the cerebrosids are *phrenosin* and *cerasin.* On hydrolysis phrenosin apparently yields cerebronic acid, galactose and sphingosin, while cerasin yields lignoceric acid, galactose and sphingosin. Thus the important difference in the two substances appears to be in the fatty acid they contain. Phrenosin has been somewhat more studied than cerasin.

Sulphatids.—It has been suggested by Koch that the oxidized sulphur always present in cerebrosids when impure has a union in the form of sulphuric acid with a cerebrosid and a phosphatid as follows:



Its nature is unknown.

Thudichum has also isolated in small amounts two amino-lipotids, *crinosin* and *bregenin.*

Cholesterol.—Cholesterol is the chief sterol present in brain. Cholesterol melts at 145°. There is another sterol present which melts at 137°,

which has been called phrenosterol. Cholesterol is present chiefly in the free state.

Extractives.—The most important nitrogenous extractives recognized are hypoxanthin, and creatin, which is present to the extent of about 0.1 per cent. Among the amino acids isolated have been tyrosin and normal leucin, or caprin. Lactic acid and inositol are also present. About 1 per cent of ash is present and this is composed in great part of alkaline phosphates and chlorids. Potassium is probably the most important base.

Cerebrospinal Fluid

Normally the cerebrospinal fluid is a perfectly clear and colorless fluid with a specific gravity of 1.005 to 1.008, and a solid content between 1 and 2 per cent. The normal amount of spinal fluid has been estimated roughly as 60 c.c., but pathologically the amount may be much larger, especially in hydrocephalus. The trace of protein present in the fluid is globulin in character. Fibrinogen and albumin are absent. The fluid is hypertonic. It is probably formed by the secretory cells covering the choroid plexus, according to recent studies of Cushing and his coworkers. Its function is unknown. It would seem probable that the secretion of the pituitary passes into the fluid. Normally not more than 3 to 5 white cells per cu. mm. of fluid are present.

From time to time many studies have been carried out on the spinal fluid, although scarcely as accurate data are available as in the case of blood, for the probable reason that the work has been carried out less systematically. In the table below are given figures for the average normal content of the various constituents in the spinal fluid, the data being taken from various sources. From the figures given it is apparent that the spinal fluid may be considered as a dialysate or ultrafiltrate of the blood plasma. It contains very little protein so long as the fluid remains normal, but nearly as much urea and glucose, and rather more salt than the blood.

In pathological cases the properties may change, particularly in meningitis. The fluid may be greatly increased in amount, under high pressure, and have a considerable increase in protein.

Denis and Ayer have presented recently some quantitative figures on the protein content of spinal fluid. Normally they found the fluid to contain from 0.04 to 0.1 per cent of protein. In active tabes, moderately active syphilis of the nervous system and lethargic encephalitis the protein content ranged from 0.1 to 0.2 per cent, in recent cerebral vascular disturbances such as hemiplegias and cerebral embolus from 0.1 to 0.3 per cent, in acute syphilis of the nervous system and general paresis from 0.2 to 0.6 per cent, while in tubercular and acute meningitis such high figures as 0.2 to 1.0 and 0.4 to 1.3 per cent respectively were observed. By taking

Composition of Normal Spinal Fluid

Determination, Recorded in	Range	Average
Total solids, per cent	0.8 – 1.6	1.0
Ash, per cent		0.88
Protein, per cent	0.04– 0.1	0.7
Nonprotein nitrogen, mg. to 100 c.c.	17.0 –26.0	21.0
Urea nitrogen, mg. to 100 c.c.	7.0 –14.0	10.0
Creatinin, mg. to 100 c.c.	0.7 – 1.5	1.0
Uric Acid, mg. to 100 c.c.	trace	0.1
Sugar, per cent	0.07– 0.1	0.08
CO_2 combining power, volumes per cent	58.0 –63.0	60.0
Chlorids as NaCl, per cent	0.60– 0.75	0.7
Phosphates as P, mg. to 100 c.c.		2.5
Sulphates as S, mg. to 100 c.c.		trace
Sodium as Na, mg. to 100 c.c.		320.0
Potassium as K, mg. to 100 c.c.	14.0 –28.0	20.0
Calcium as Ca, mg. to 100 c.c.		7.0
Magnesium as Mg, mg. to 100 c.c.		3.0
pH (when first drawn)		7.4
pH (on standing)		8.3

advantage of the changed reaction of the fluid in the last mentioned conditions and the rate of change of alkalinity on standing, Tashiro and Levinson have devised a very valuable method of differentiating tubercular from epidemic meningitis. If to 1 c.c. of spinal fluid there is added 1 c.c. of 3 per cent sulphosalicylic acid, and to another 1 c.c. of fluid a like amount of 1 per cent mercuric chlorid, then in tubercular meningitis the protein which settles down on standing 24 hrs. is more voluminous in the mercury tube, whereas in epidemic meningitis it is more voluminous in the sulphosalicylic acid tube.

The nonprotein nitrogen of spinal fluid averages only about 70 per cent of the figures obtained in blood, but this statement does not apply to its chief component, urea. It is now well known that the various membranes of the body are very permeable to urea, resulting in an even distribution of this waste product throughout the tissues of the body, as shown by Marshall and Davis. Cullen and Ellis have strikingly pointed this out in the case of spinal fluid. Myers and Fine(*n*) likewise have found this to be true in nephritis with marked nitrogen retention. In their series of fifteen cases the spinal fluid urea averaged 88 per cent of that of the blood. The concentration of creatinin averaged 46 per cent of that found in the blood in the same series, indicating that it did not diffuse as readily as the urea. In one case with the high blood creatinin of 14.5 mg., the spinal fluid content was 4.8, while in a similar case the figures were 11.0 and 4.2 mg. respectively. Uric acid does not readily pass into the spinal fluid, if one is to judge from observations on the same cases, since the amount present averaged only about 5 per cent of that found in the blood. In a few exceptional cases the figures for the spinal fluid reached only about 1 mg., and this despite the fact that the blood content was about 10 mg.

The sugar normally amounts to 0.07 to 0.09 per cent, in comparison with figures of 0.09 to 0.11 per cent for the blood. Sugar appears to be fairly readily admitted to the spinal fluid, since in diabetes comparatively high figures may be found. Myers and Fine(*n*) observed a sugar content of 0.30 per cent in a case of diabetes showing a blood sugar of 0.44 per cent. In meningitis the sugar content may be either very low or entirely absent, negative findings more often being observed in epidemic and pneumococcus meningitis than in tubercular meningitis. The estimation of the sugar in meningitis may therefore be of considerable practical value.

The CO_2 combining power of spinal fluid averages 60 volumes per cent, which is slightly lower than that of normal blood plasma. It likewise seems to vary within narrower limits.

Of the mineral constituents of the spinal fluid the chlorids are by far the most significant in point of quantity. Calculated as NaCl the chlorids normally appear to average 0.7 per cent, more than half of the total solid content. The content is considerably greater than that of the blood plasma. It is ordinarily stated to be hypertonic to lymph, but theoretically it would seem more likely that the high content of salt was required to render this fluid isotonic with the blood. The chlorid content of the spinal fluid is apparently increased in those conditions in which an increase is found in the blood.

The phosphates of the spinal fluid, which normally amount to about 2.5 mg. per 100 c.c., calculated as P, are increased (8-10 mg.) in certain mental disorders, notably paresis. In view of the importance attached at the present time to the increase in the inorganic phosphates of the blood in nephritis with acidosis, it may be of interest to note that Myers in 1909 observed a P content of 19 mg. in the spinal fluid of a patient dying from "arteriosclerosis." In view of the close relation of both phosphoric acid and cholin in lecithin, note may be made regarding cholin at this time. The presence of cholin in the spinal fluid of paretic patients was first claimed by Mott and Halliburton, and confirmed by a number of workers in this and other conditions involving nerve degeneration. Later, however, the presence of cholin was disputed.

The metallic elements, sodium, potassium, calcium and magnesium, with the exception of the first named, apparently exist in the spinal fluid in practically the same concentration as in the blood. Sodium appears to be present in somewhat larger amounts as the high chlorin content of the fluid would indicate. Some years ago Rosenheim reported that potassium was present in relatively large amounts in cases of acute degenerative insanity where cholin was present. In reinvestigating this question Myers (*b*) found that the potassium content of the fluid in dementia paralytica and several other conditions during life averaged 20 mg. per 100 c.c., but that after death the figures amounted to slightly more than

80 mg., indicating that the high figures for potassium were due to post-mortem causes and possessed no pathological significance. This post-mortem increase is quite striking, however, since as high figures are found one-half hour post mortem as at any other time. This very rapid post-mortem rise in the potassium is significant. The findings for calcium and magnesium differ little from those obtained in blood. Levinson(*b*) has found that the pH determined immediately on withdrawing the fluid varied between 7.4 and 7.6. It was normal in all pathological conditions observed, except epidemic meningitis, where figures of 7.3 to 7.4 were generally observed.

Saliva

Mixed human saliva is composed of the secretion of three pairs of glands, the submaxillary, sublingual and parotid, supplemented by the secretion of numerous small glands called buccal glands. The saliva secreted by the different pairs of glands possesses different characteristics, the secretion of the parotid being thin and watery, while that of the sublingual and submaxillary, particularly the former, is thick and viscid, owing to the large amount of mucin present. The amount of saliva secreted by an adult in twenty-four hours has been variously estimated as between 1000 and 1500 c.c., the exact amount depending, among other conditions, upon the character of the diet. The specific gravity varies between 1.002 and 1.008, with an average of 1.005.

According to Frerichs mixed saliva has the following composition:

Composition of Human Saliva

Constituents	In Per Cent
Water	99.41
Solids	0.59
Mucin and epithelium	0.213
Soluble organic matter	0.142
Inorganic salts	0.219
Potassium thiocyanate	0 to 0.010

Normally saliva is alkaline to litmus and acid to phenolphthalein, the reaction being practically the same as that of the blood. The chief constituents of the ash are potassium, phosphate and chlorids, which together constitute about 80 per cent of the mineral content.

The important organic constituents of the saliva are the mucin (a glycoprotein) and the salivary amylase, ptyalin, the former aiding in swallowing and the latter in the digestion of starch. At one time it was argued that ptyalin could be of little value in starch digestion since it was probably destroyed by the hydrochloric acid of the gastric juice as soon as it reached the stomach. It has been shown by Cannon, however,

that salivary digestion may proceed for a considerable period after the food reaches the stomach, owing to the slowness with which the food contents are mixed with the acid gastric juice. Ptyalin acts best in a neutral or faintly acid medium (combined acid), but is readily destroyed by a trace of free hydrochloric acid. It acts more efficiently when somewhat diluted.

It has been shown by Chittenden and Smith that the diastatic action of human saliva can be taken as a definite measure of the amount of ferment present, only when the saliva in the digestion mixture is diluted at least 50 or 100 times. They have found that the limit of dilution at which decisive diastatic action manifests itself with formation of reducing bodies is 1 to 2000 or 3000. Myers and Dellenbaugh, working with a very delicate method, have recently observed that when 0.01 c.c. of normal human saliva is allowed to act on 10 mg. of soluble starch in a volume of 2 c.c. for 30 minutes at 40° C., 30 to 45 per cent of the starch is converted to sugar when the diluent is water and 46 to 50 per cent when the diluent of the saliva is 0.3 per cent sodium chlorid. The Cl ion has long been recognized to have a pronounced facilitating action. Essentially the same range of figures were found in such pathological conditions as diabetes, nephritis and gastric ulcer. A few individuals were encountered, however, who for periods showed low activities, figures 10 to 20, that were not readily explained, although it may be noted that they complained of gastric distress. Representatives of different nationalities were found to vary within the same normal limits, which opposes the view advocated by some of the adaptation of salivary secretion to diet. As shown by Chittenden and Richards, saliva secreted after a period of glandular activity, as before breakfast, manifests greater amylolytic power than the secretion obtained after eating. Corresponding with this increase in amylolytic power occurs an increase in the proportion of alkaline-reacting salts, but the increased amylolysis is due primarily to an increase in the amount of active enzyme contained in the saliva.

Marshall has suggested that the ratio between the mathematical expressions for the total neutralizing power of normal resting saliva and normal activated saliva from a given individual is a "salivary factor" the magnitude of which appears to be indicative of immunity from caries or the reverse. Shepard and Gies were unable to substantiate this claim.

The thiocyanate content of human saliva has been the topic of a number of studies. The saliva of smokers has been shown to have a much higher content of potassium thiocyanate than that of nonsmokers. Schneider found that the average content for six smokers was 0.013 per cent, while for ten nonsmokers it was 0.003 per cent. Sullivan and Dawson have studied the thiocyanate content of the saliva in pellagra. With active symptoms the thiocyanate content is lower than later when the characteristic symptoms have disappeared. The thiocyanate content of

eighteen patients on admission averaged 0.0035 per cent, while on discharge it was 0.0047 per cent.

Milk

Milk is a product of the secretory activity of the mammary gland. It is the most satisfactory food material elaborated by nature. As a food it is deficient in only one respect, viz., its iron content. This is without significance when milk is used as a food for infants, since a considerable quantity of iron is stored up in the liver during fetal life. Milk contains the proteins, casein and lactalbumin, such fats as olein, palmitin, stearin and butyrin, the disaccharid, lactose, together with phosphates of calcium, potassium and magnesium, citrates of sodium and potassium, and chlorid of calcium. In addition it is evident from recent observations that milk is well supplied with the water soluble and fat soluble vitamins, together with a sufficient quantity of the antiscorbutic element.

The physical appearance of milk suggests that the various constituents are not all in solution. Fat is present in a finely divided suspension, while casein is either in suspension or in a colloidal solution. Van Slyke and Bosworth have been able to separate the insoluble portion of milk by filtration through a Pasteur-Chamberland filter. With the aid of this method they have been able to divide the constituents of milk into three groups as shown by the table below:

Milk Constituents

In True Solution in Milk Serum	Partly in Solution and Partly in Suspension or Colloidal Solution	Entirely in Suspension or Colloidal Solution
Lactose	Lactalbumin	Fat
Citric acid	Inorganic phosphates	Casein
Potassium	Calcium	
Sodium	Magnesium	
Chlorid		

Perfectly fresh milk, both human and cow's, is amphoteric in reaction toward litmus and acid to phenolphthalein. The acidity to phenolphthalein is due in considerable part to acid phosphates, although acid caseinates may be responsible for some of the acidity. The specific gravity of milk most often varies between 1.028 and 1.032. Milk has a very slight yellow color, which is more noticeable in the cream on standing. The yellow pigments of butter fat are the vegetable pigments carotin and xanthophylls. They are present in the colostrum in much higher concentration than in mature milk.

The milk of different species of animals differs very materially, the animals with a rapid rate of growth secreting a milk with a much higher

content of protein and salts and a somewhat lower lactose content. The following table, compiled largely from analyses made in Bunge's laboratory, nicely illustrates this point:

RATE OF GROWTH AND COMPOSITION OF MILK

Species	Number of Days Required to Double Weight at Birth	Percentage Composition of Milk		
		Protein	Ash	Lactose
Human	180	1.6	0.2	7.0
Horse	60	2.0	0.4	6.7
Cow	47	3.5	0.7	4.9
Goat	22	3.7	0.8	4.4
Sheep	15	4.9	0.8	4.0
Swine	14	5.2	0.8	4.0
Dog	9	7.4	1.3	3.2
Rabbit	6	10.4	2.5	...

Holt, Courtney and Fales have recently made a quite elaborate study of the composition of human milk. A summary of some of their results is given in the table below. As will be noted in the colostrum period human

PERCENTAGE COMPOSITION OF HUMAN MILK BY PERIODS

Period	No. of Analyses	Fat	Sugar	Protein	Casein	Albumin	Ash	Total Solids
Colostrum (1-12 days)	5	2.83	7.59	2.25	.....	.....	0.31	13.4
Transition (12-30 days)	6	4.37	7.74	1.56	.....	.....	0.24	13.4
Mature (1-9 mos.)	17	3.26	7.50	1.15	0.43	0.72	0.21	12.2
Late (10-20 mos.)	10	3.16	7.47	1.07	0.32	0.75	0.20	12.2

milk has a high protein and high ash with rather low fat, in the transition period the protein and ash are lower while the fat is higher, but after one month the composition of normal milk does not vary in any essential or constant way quite up to the end of lactation. The only striking feature of late milk is a decline in quantity, though there is noted a slight fall in all the solid constituents except the sugar. Of the different constituents of milk, the least variation in both individuals and periods is seen in the sugar. It will be observed in the table that the sugar amounts to about 7.5 per cent, which is higher by a half per cent than the generally accepted figure of 7 per cent. The greatest individual variations are observed in the fat (figures from 1 to 6 per cent), although as recorded above, the period variations in the fat are not marked. The protein is highest in the colostrum period and falls to a little over half the proportion in mature milk, during which period it is seldom over 1.25 per cent; of this about one-third is casein and two-thirds lactalbumin.

Meigs and Marsh give the following table as representative of the

limits of normal variation in the constituents of human and cow's milk from the beginning of the second month of lactation onward, the figures representing percentages of whole milk:

	Fat	Lactose	Protein
Human milk	2–4	6–7.5	0.7–1.5
Cow's milk	2–4	3.5–5.0	2.5–4.0

It is apparent that human milk contains less protein but more sugar than cow's milk. The protein of human milk differs from that of cow's milk in one very important respect, quite aside from the total quantity of protein. It contains much less casein but rather more lactalbumin. According to Meigs and Marsh, both human and cow's milk contain important non-nitrogenous substances of an unknown character. Early human milk contains about 1 per cent of these unknown substances; milk from the middle period of lactation about 0.5 per cent. Cow's milk from the middle period of lactation contains about 0.3 per cent of the unknown substance.

Denis, Talbot and Minot have studied the nonprotein nitrogenous constituents of human milk. They summarize the results of the examination of 71 samples as follows:

Nonprotein Nitrogenous Constituents	mg. to 100 c.c.	
	Minimum	Maximum
Total nonprotein nitrogen	20.0	37.0
Urea nitrogen	8.3	16.0
Amino nitrogen	3.0	8.9
Preformed creatinin	1.0	1.6
Creatin	1.9	3.9
Uric acid	1.7	4.4

In some of the cases the nonprotein and urea nitrogen were also determined in the blood and practically the same figures obtained as in the milk.

In a series of about forty cases Denis and Minot(*c*) found the cholesterol content of human milk to vary from 10 to 30 mg. per 100 c.c., figures of 10 to 20 mg. being obtained chiefly in milk with a low fat content and figures of 20 to 30 mg. with a high fat content. According to Bosworth and Van Slyke, cow's milk contains 0.052 per cent of potassium citrate and 0.222 per cent of the sodium salt, while in human milk the potassium salt, 0.103 per cent, is in excess of the sodium salt, 0.055 per cent. Sommer and Hart have shown that the citric acid of cow's milk (0.2 per cent) is not destroyed or changed on heating.

The mineral content of milk is of great interest and importance. Holt, Courtney and Fales(*b*) have given the average composition of the

ash of human milk for different periods and also for cow's milk, their figures being given in the table below. As will be noted the high ash of

AVERAGE PERCENTAGE COMPOSITION OF THE ASH OF HUMAN AND COW'S MILK

		CaO	MgO	P_2O_5	Na_2O	K_2O	Cl
Human	Colostrum	14.2	3.5	12.5	13.7	28.1	20.6
	Transition	17.0	2.4	16.9	10.9	30.8	22.9
	Mature	23.3	3.7	16.6	7.2	28.3	16.5
	Late	19.8	3.6	15.5	10.1	28.8	22.3
Cow's milk		23.5	2.8	26.5	7.2	24.9	13.6

the colostrum period is due chiefly to the amount of Na_2O and K_2O. Of the salts which make up the ash, the greatest individual, as well as the greatest period, variations are seen in the Na_2O. The largest constituent of the ash of human milk is K_2O, this with the CaO together making up more than half the total ash. Although in amount the total ash of cow's milk is about three and one-half times as great as that of human milk, the proportion of different salts which make up the ash is nearly the same, the only exception being that cow's milk has more P_2O_5 and less iron.

SECTION IV

Excretions

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There are four mediums for the excretion of waste products from the body, viz., urine, feces, perspiration and expired air. Under normal conditions and on a readily digestible diet, nearly 100 per cent of the carbohydrate, about 95 per cent of the fat and more than 90 per cent of the protein—if no correction is made for the "metabolic nitrogen" of the feces—are completely digested and absorbed. The carbohydrate and fat absorbed are almost entirely converted to carbon dioxid and water, and this is also true of the carbon moiety (about 80 per cent) of the protein. The carbon dioxid thus formed is excreted by way of the lungs, as is a large amount of the water in the form of water vapor. Considerable water may be lost from the body by way of the perspiration but the amount of solids excreted in this way is never large, although with severe exercise and sweating from 0.3 to 0.5 gram of nitrogen and 0.5 to 1.5 grams of sodium chlorid may be eliminated. The chief paths for the excretion of solids are the kidney and intestine, the daily elimination by these two channels in the adult amounting to about 50 grams in the urine and 30 grams in the feces. The nitrogenous waste products find their principal exit through the kidneys, but in the case of the mineral elements the kidneys and intestines both take part, the salts of sodium and potassium being largely eliminated in the urine, while the salts of calcium, magnesium and iron are excreted in the feces. Although the excretion of the latter compounds in the feces may be due in part to lack of absorption, still there is likewise a definite selective action regarding their excretion. An excellent illustration of how changes in compounds may affect their mode of excretion is the elimination of the two phenolphthalein derivatives, phenolsulphonephthalein and tetrachlorphthalein. The former is eliminated entirely by the kidneys, while the latter after being secreted in the bile by the liver is excreted by way of the intestines.

Urine

Since the end products of the metabolism of nitrogenous and mineral substances find their principal exit through the kidneys, a study of the

secretion of these glands under various conditions may be expected to throw light upon the processes involved in the metabolism of the above substances. With a knowledge of the principal constituents of the urine and a partial understanding, at least, of their history in the body, the appearance of any unusual substance or the presence of a normally occurring constituent in an amount inconsistent with the attending conditions may bring to light derangements of body functions.

The mechanism of kidney secretion has been a much controverted question. The view (modified Heidenhain) which has been most generally held for some years past is that the renal cells actively participate in the secretion, the water and inorganic salts being eliminated in the capsular region, while the urea, creatinin, uric acid, etc., find their exit through the uriniferous tubules. Quite recently our conception of urine secretion has undergone material change partly as a result of advances in our knowledge of physical chemistry and partly from added anatomical data. From a study of the blood vessel structure of the glomerulus, it is apparent that the blood pressure in the glomerular capillaries must be high, much higher than that of the fluid in the capsule. According to the "modern theory" (Cushny (*b*)), the secretion of urine consists of two distinct processes differing not only in site but also in nature. The first of these, the filtration, occurs in the glomerulus, and is purely physical; the second, the reabsorption, occurs in the tubules, and depends upon the vital activity of the epithelium. By the first process the protein colloids of the blood plasma are filtered off. By the second process water and so-called threshold bodies such as chlorids and sugar are largely reabsorbed, while no-threshold substances, such as urea, are rejected and can only escape by the ureter.

That the cells of the tubules actively participate in the secretion of urea, however, seems apparent from recent experiments of Oliver. With the aid of xanthydrol he has shown that urea is present in the cells of the proximal convoluted tubules in a concentration higher than that of the blood or that of the cells of any of the other kidney tubules, which condition can only be reconciled to an assumption of an active secretion (excretion) on the part of these cells.

Physical Properties.—*Volume.*—The volume of urine eliminated depends in great part upon the volume of fluid ingested. Under normal conditions 1000 c.c. may be taken as the average volume of urine excreted in 24 hrs. This, however, is subject to great variations under both normal and pathological conditions.

The volume of urine is diminished by conditions which cause an increased elimination of water through other channels, for example through the alimentary tract during diarrhea and vomiting, or through the skin as perspiration. On the other hand during cold weather, when cutaneous evaporation is reduced, the volume of urine is increased. Thus

in warm weather the volume may be as low as 350 c.c., while a volume of 1500 to 1800 c.c. may be encountered during cold weather.

The condition of the cardiovascular system and kidneys has much to do with the volume of urine eliminated. In interstitial nephritis, the volume of urine is usually large, frequently 2000 c.c. or over. Of particular interest is the observation that in this condition an abnormally large volume of dilute urine is eliminated during the hours from 8 P.M. to 8 A.M. This night polyuria commonly results in an elimination considerably in excess of 400 c.c., the usual output during these hours. In parenchymatous nephritis, the relations are not so constant, but in general the urine is concentrated and the volume reduced. The variations in volume in such cases are usually referable to the formation or disappearance of edema. A very large volume of dilute urine (5000 c.c. or more) may be eliminated in diabetes insipidus, due probably to dilatation of the renal vessels. The volume is increased when it is necessary to eliminate a large amount of material, as is the case with sugar in diabetes mellitus. A temporarily increased output of urine may result through nervous influences.

Color.—The color of urine may vary under normal conditions from a very pale yellow to a reddish yellow or deep amber, depending upon its density. The color is due principally to a pigment called urochrome, although small amounts of urobilin, and occasionally traces of uroerythrin may be present. Pathologically the color may vary from a perfectly colorless fluid to dark brown or black. A red color may be due to blood, occasionally to hematoporphyrin; very dark colored urines may arise from taking carbolic acid; the excretion of melanin from pigmented tumors may likewise be the cause of a dark color, especially after being exposed to the air for some time or on the addition of an oxidizing agent. A green or brownish yellow color may be due to bile, also recognized by the yellow tinged foam. In alkaptonuria the urine may become dark owing to the presence of homogentisic acid. This is especially so if the urine is allowed to become alkaline.

Specific Gravity.—The specific gravity of normal urine most commonly falls between 1.015 and 1.025. It may, however, be as low as 1.008 or as high as 1.040 without necessarily indicating pathological conditions. Normally the specific gravity is inversely proportional to the volume. In diabetes mellitus one may observe both a large volume and a high specific gravity owing to the presence of sugar. In interstitial nephritis the specific gravity is persistently low and fixed regardless of variations in volume.

Odor.—Normal urine has a faint but characteristic aromatic odor. As urine undergoes alkaline fermentation, a disagreeable ammoniacal odor develops.

Reaction and Acidity.—The principal factor involved in the regula-

tion of urinary acidity is the proportion between the acid sodium phosphate (H_2NaPO_4) and the basic sodium phosphate (HNa_2PO_4), the former raising the acidity and the latter lowering it. The principal acid supply is found in the metabolism of protein, during which sulphuric acid is formed from the oxidation of the sulphur of the protein, while phosphoric acid is set free. The organic acids, uric, hippuric, oxalic, and certain of the lower fatty acids also contribute to the acidity. The basic radicals concerned are sodium, potassium, ammonium, calcium and magnesium. The excretory function of the kidney normally prevents any undue accumulation of either acids or bases in the body, thereby maintaining a remarkable constancy in the reaction of the body fluids.

Urine is most commonly acid to litmus. The reaction and degree of acidity may, however, experience marked change under both physiological and pathological conditions. The diet is one of the most important factors involved. In general, the metabolism of animal foods, except milk, results in an increased acidity, while vegetable foods, except the cereal grains, tend to diminish the acidity or even yield alkaline urines. The reason for this general difference between animal and vegetable food materials is due, as pointed out by Sherman and Gettler, to their excess of acid- or base-forming elements. These considerations probably account for the fact that the urine of dogs is normally acid, while that of rabbits is habitually alkaline.

The pathological formation of acids (as in diabetes) is counteracted in a measure by the neutralizing action of the bases, sodium, potassium, calcium and magnesium. When the acidity is so great that an adequate supply of these elements can no longer be economically furnished by the body, ammonia is called upon to meet this need. This accounts for the increased elimination of ammonia in diabetic ketosis. The proximity to a meal may affect the reaction of the urine. For example, the secretion of hydrochloric acid in the stomach during the process of digestion may so reduce the store of acids in the body that for a time after a meal the urine may be neutral or even alkaline, giving rise to the so-called "alkaline tide."

Quantitative expression may be given to the acidity of the urine by determining the number of cubic centimeters of tenth normal sodium hydroxid required to neutralize the total volume of urine eliminated in 24 hrs. This represents the *titratable* acidity, and may range from 200 to 500, with an average of about 350.

The *titratable acidity* should be distinguished from the *true acidity*, the latter depending upon the concentration of ionized hydrogen (H^+). From this point of view, a solution is acid, neutral or alkaline, depending upon the relative concentrations of hydrogen ions (H^+) and of hydroxyl ions (OH^-). An acid solution therefore contains a greater concentration of (H^+) than of (OH^-). For convenience in recording the hydrogen ion

concentration a simplified logarithmic notation is generally employed. Pure water, our standard of neutrality, contains $\frac{1}{10{,}000{,}000}$ of a gram of H^+ to a liter, and is therefore a $\frac{1}{10{,}000{,}000}$ N solution of H. For convenience the logarithmic notation is employed, thus: $\frac{1}{10{,}000{,}000\ N} = \frac{1}{(10)^7\ N} = 10^{-7}$. Since the base is always 10, and the logarithm always negative the expression is further simplified by dropping both the figure 10 and the minus sign. The hydrogen ion concentration of pure water, then, is expressed in terms of its exponent, pH = 7. Since the sum of the logarithmic expressions H and (OH) ion concentrations is always 14, it will be readily seen that the concentration of either ion may be estimated when one is known. In practice the determination of the hydrogen ion has been found simpler.

Normally the urine appears to vary from an acid solution of pH = 4.82 to an alkaline solution of pH = 7.45, the average being close to a solution of pH = 6.0. By the administration of sodium bicarbonate and sodium citrate (which is oxidized to the carbonate), Henderson and Palmer(*a*) were able to lower the pH to 8.70, a condition of alkalinity. As pointed out by Blatherwick(*a*) foods yielding basic ashes may likewise reduce the urinary acidity to that of neutrality (pH = 7), or even beyond this to alkalinity. Among 30 vegetarians the pH varied from 5.30 to 7.48, averaging 6.64. Palmer and Henderson(*b*) have shown that in cases with cardiorenal diseases, the acidity of the urine is usually increased. The average pH of 57 cases was 5.33, representing a five-fold increase in urinary acidity over the normal average of 6.0.

Transparency.—When voided the urine of a normal individual is usually perfectly clear. On standing a few hours a cloud or "nubecula" forms, even in normal urine. This cloud consists of mucus threads, epithelial cells, etc., from the urinary passages. Under pathological conditions, the latter may be greatly increased and accompanied by casts or blood. If the acidity of the urine is somewhat diminished (as after a meal) a turbidity due to phosphates will form. This will disappear on adding a little acetic acid. On standing in the cold, urates may settle out but will again go into solution on warming.

Organic Constituents

By far the greater number of organic compounds present in normal urine contain nitrogen, and those that do not contain nitrogen constitute an extremely small part of the total solids. Fifty grams may be given as a rough figure for the solid content of urine and of this amount about

60 per cent is ordinarily organic and the remainder inorganic. Since the organic constituents of urine are chiefly nitrogenous and since the nitrogenous waste products are eliminated principally in the urine, i.e., to the extent of 85 to 90 per cent, a study of their elimination in the urine under different conditions of diet should furnish considerable insight into the controlling factors in protein metabolism.

The most satisfactory discussion of this subject has been given by Folin(*b*) in his now classic papers published in 1905. With the aid of many new methods which he had developed, Folin found it possible to make fairly complete analyses of single 24 hr. specimens of urine. By a study of the comparative distribution of the nitrogenous compounds in the urine on two diets, one containing rather more than 100 grams of protein and the other (starch-cream) containing about 1 gram of nitrogen, he was able to differentiate between the *endogenous* and *exogenous* origin of the different waste products. As a result of these observations he evolved a new theory of protein metabolism, which quickly supplanted the untenable theories of Pflüger and Voit.

The important components of the total nitrogen of the urine are the nitrogen of the urea, creatinin, ammonia and uric acid. The following data taken from Folin illustrate the distribution of these compounds (likewise the various sulphur compounds which are also derived from the protein) in the urine of the same individual on a high and on a low protein diet.

	Normal Protein Diet July 13	Low Protein Diet July 20
Volume of urine	1170 c.c.	385 c.c.
Total nitrogen	16.80 gm.	3.60 gm.
Urea nitrogen	14.70 " = 87.5%	2.20 " = 61.7%
Ammonia nitrogen	0.49 " = 3.0	0.42 " = 11.3
Uric acid nitrogen	0.18 " = 1.1	0.09 " = 2.5
Creatinin nitrogen	0.58 " = 3.6	0.60 " = 17.2
Undetermined nitrogen	0.85 " = 4.9	0.27 " = 7.3
Total SO_3	3.64	0.76
Inorganic SO_3	3.27 " = 90.0	0.46 " = 60.5
Ethereal SO_3	0.19 " = 5.2	0.10 " = 13.2
Neutral SO_3	0.18 " = 4.8	0.20 " = 26.3

From the above data it is apparent that the distribution of the nitrogen in the urine among urea and the other nitrogenous constituents depends on the absolute amount of total nitrogen present (the distribution of the sulphur likewise being dependent upon the amount of the total sulphur). As will be noted urea is the only nitrogenous substance which suffers a relative as well as an absolute diminution with a decrease in the total protein metabolism. On the other hand, as Folin was the first to point out, the absolute quantity of creatinin eliminated in the urine on a meat free diet is a constant quantity, different for different individuals,

but wholly independent of quantitative changes in the total amount of nitrogen eliminated. It may be observed in the case of the uric acid that when the total amount of protein metabolism is greatly reduced, the absolute quantity of uric acid is diminished, but not nearly in proportion to the diminution in the total nitrogen, and the per cent of the uric acid nitrogen in terms of the total nitrogen is therefore much increased. From these observations Folin pointed out that urea and creatinin stand in marked contrast to each other, since the former is largely *exogenous* in origin, while the latter is almost entirely of *endogenous* formation. Uric acid stands in an intermediate position, being about half endogenous and half exogenous under ordinary conditions of diet.

Since urea is largely exogenous in its origin the amount of its excretion in the urine obviously depends upon the protein intake. With the dietary standards of Voit and of Atwater calling for 118 to 125 grams of protein, the urea output should be 30 to 35 grams. Comparatively few healthy adults appear to eliminate as much urea as this at the present time. Probably 25 grams may be taken as more nearly representing the average output of urea in the human adult, although judging from the very extensive data given in the Referee Board reports, many individuals average hardly more than 20 grams, corresponding to a protein intake of 75 to 80 grams. It is obvious, therefore, that the daily excretion of 10 to 15 grams of urea by many hospital patients finds explanation as a rule, not in defective kidney function, but in a low protein intake. Even here the urea excretion represents a protein consumption of 40 to 60 grams, an amount which Chittenden(*b*) has shown may quite adequately supply the requirements of the average individual.

Assuming that the average urea output of the human adult is 25 grams, the content of the various nitrogenous constituents with their distribution in the total nitrogen may be represented as given in the table below. With this output of urea the urea nitrogen would probably constitute about 85 per cent of the total nitrogen, thus making the figure for

AVERAGE CONTENT OF THE NITROGENOUS CONSTITUENTS IN THE URINE OF THE HUMAN ADULT

Constituent	Weight of Substance	Nitrogen Equivalent	Relation of Nitrogen Equivalent to Total Nitrogen
	Grams	Grams	Per Cent
Total nitrogen		13.8	100.0
Urea	25.0	11.7	85.0
Ammonia		0.5	3.6
Creatinin	1.5	0.56	4.1
Uric acid	0.5	0.17	1.6
Undetermined N		0.79	5.7
Hippuric acid	0.7	0.06	
Amino acids	0.5	0.10	
Purin bases	0.03	0.01	

total nitrogen 13.8 grams. If allowance is made for a fecal nitrogen excretion of 1.5 grams, the nitrogen intake would be 15.3 grams, which corresponds to about 95 grams protein. The output of creatinin for the average human adult is about 1.5 grams and of uric acid 0.5 gram.

Urea.—Urea, ammonia and amino acids are intimately related in their physiological history. It will be recalled that the amino-acids, resulting from the digestion of protein in the intestine, are absorbed and carried to all the tissues of the body. The greater part of the amino-acids thus absorbed and disseminated are deaminized, i.e., the amino group (NH_2) is split off, forming ammonia. This process of deamination may be illustrated as follows, taking alanin as a typical amino-acid:

$$\underset{\text{Alanin}}{CH_3.\overset{\overset{NH_2}{|}}{CH}.COOH} + \underset{\text{Water}}{HOH} \rightarrow \underset{\text{Ammonia}}{NH_3} + \underset{\text{Lactic Acid}}{CH_3.\overset{\overset{OH}{|}}{CH}.COOH}$$

The ammonia unites with the carbonic acid of the blood and tissues to form ammonium carbonate. Two molecules of water are then split off from the ammonium carbonate, yielding urea. The formation of ammonium carbonate and its subsequent dehydration to form urea are indicated below:

$$\underset{\text{Carbonic acid}}{\begin{matrix} OH \\ | \\ C=O \\ | \\ OH \end{matrix}} + \underset{\text{Ammonia}}{\begin{matrix} NH_3 \\ \\ \\ \\ NH_3 \end{matrix}} \rightarrow \underset{\text{Ammonium Carbonate}}{\begin{matrix} ONH_4 \\ | \\ C=O \\ | \\ ONH_4 \end{matrix}} \rightarrow \underset{\text{Urea}}{\begin{matrix} NH_2 \\ | \\ C=O \\ | \\ NH_2 \end{matrix}} + \underset{\text{Water}}{\begin{matrix} H_2O \\ \\ \\ \\ H_2O \end{matrix}}$$

Kossel and Dakin have also shown that arginin may be directly split into ornithin and urea under the action of a liver enzyme, arginase. The deamination of amino-acids and the transformation of ammonium carbonate into urea takes place in the liver and possibly in other tissues. (See preceding article, p. 464.) Because of the prominence played by the liver cells in these processes, considerable importance has been attached to apparent abnormalities in the elimination of urea, ammonia and amino-acids. In acute yellow atrophy of the liver, interstitial hepatitis and cirrhosis of the liver, there is a very extensive degeneration of the liver cells. The association of hepatic disturbance with increased elimination of ammonia and amino-acids, and diminished output of urea has not been a constant finding (Fiske and Karsner), and in many instances has been the result of employing old and inadequate methods. However, there is

generally some reduction in the amount of urea in the urine, and an increase in the ammonia content.

Urea is an extremely soluble and relatively non-toxic substance. These two properties have a particular significance in view of the fact that urea is the chief end product of protein metabolism, and is almost wholly eliminated through the kidney, the portion excreted through other channels such as the skin being relatively unimportant. The quantitative output of urea is closely proportional to the amount of protein ingested. Variations of 10 to 40 grams may be encountered in perfectly normal individuals. The percentage of urea is dependent upon the volume of urine in addition to the protein of the diet, and when it is considered that the former may vary from 500 to 2000 c.c. it is evident that but little information concerning the quantity eliminated can be gained from a knowledge of merely the percentage of urea. The urea nitrogen in proportion to the total nitrogen excreted may likewise be greatly influenced by the amount of protein in the diet. Thus with a high protein intake, the urea nitrogen may make up as much as 90 per cent of the total nitrogen; while with a diet containing relatively little protein but considerable carbohydrate and fat, the proportion may be as low as 60 per cent. (See table on p. 486.) With a nitrogen intake of 20 grams the urine would contain approximately 18 grams of nitrogen, of which about 16 grams would be in the form of urea; whereas with a nitrogen intake of 7 grams the excretion of urea nitrogen may be as low as 4 grams. An average quantitative output of urea with its nitrogen equivalent and the relation of the latter to the total nitrogen output is given in the table on page 487. It will be readily seen that it is quite essential in considering the excretion of total nitrogen and urea to compare these values with the nitrogen of the food, because only when the nitrogen output is out of proportion to the intake can an abnormal condition be presumed to exist.

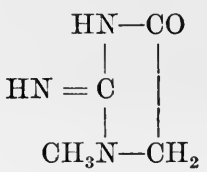
When the rate of metabolism is accelerated as in fevers, exophthalmic goiter, etc., or by the consumption of large amounts of protein as in diabetes, the total nitrogen and urea may be greatly augmented. Although the function of excreting urea may be much impaired in nephritis a recognition of this fact simply from the output of urea is difficult. Information in this regard may be more readily secured from an analysis of the blood.

Ammonia.—Under ordinary conditions the nitrogen of ammonia, in combination with urinary acids, is present in the urine to the extent of 2.5 to 4.5 per cent of the total nitrogen eliminated, i.e., about 0.5 gram per day. A considerable portion of this probably represents urea which has been reconverted into ammonia so that it might be utilized to neutralize the sulphuric, phosphoric, uric acid, etc., formed in the process of normal metabolism or introduced with the food. This procedure probably operates to

prevent undue strain upon the body's supply of sodium, potassium, calcium and magnesium. As shown by Janney(*b*), if sufficient fixed alkalies or alkali-earths are administered, so that ammonia is not required for neutralizing the acids, then the ammonia excretion may be greatly reduced, or in fact almost completely disappear from the urine. On the other hand, the ammonia output may be greatly increased when there is an abnormal acid production, as occurs in severe diabetes. Sherman and Gettler have demonstrated that the ammonia output is dependent to a considerable extent upon the balance between the acid-forming and base-forming elements of the foods. Increased elimination of ammonia has been observed in pernicious vomiting of pregnancy, but it is important to note that here the individual is essentially in a condition of inanition, which itself is characterized by a relative increase in ammonia elimination.

Amino-Acids.—Small amounts of amino-acids normally escape deaminization and appear in the urine. They represent about 0.5 per cent of the total nitrogen, and, unless specifically determined, are recorded as undetermined nitrogen. In severe liver disease, i.e., yellow atrophy, phosphorus poisoning, the output of amino-acids, may be increased, and occasionally certain amino-acids, such as leucin and tyrosin, actually crystallize out in the urine. As already noted, however, increased amino-acid excretion and hepatic disturbances are not constantly associated. In certain individuals the amino-acid cystin is eliminated in considerable amounts. This is regarded as an anomaly of protein metabolism.

Creatinin.—Creatinin is the anhydrid of creatin. It is the second largest nitrogenous constituent of urine, the daily elimination in the healthy human adult ordinarily varying between 1 and 2 grams.



Our accurate knowledge with regard to the elimination of creatinin dates from the introduction of the Folin colorimetric method in 1904. As the result of his original studies on the elimination of creatinin, Folin considered the excretion of this substance from the standpoint of a new theory of protein metabolism. He was the first to point out that the amount of creatinin excreted in the urine on a meat free diet is quite independent of either the amount of protein in the food or of the total nitrogen in the urine, the amount excreted from day to day being practically constant for each individual, thus pointing conclusively to its endogenous origin. The constancy of this creatinin excretion has been

fully confirmed by many subsequent investigators and Shaffer(*a*) has further observed the same uniformity in its hourly excretion. (According to Neuwirth the hourly creatinin excretion is generally slightly decreased during one hour in the later afternoon or early evening). Even a considerable diuresis has little effect on this hourly output, while a great increase or decrease in the amount of total nitrogen excreted per hour is likewise without effect. Furthermore, neither increased nor decreased muscular activity, uncomplicated by other factors, has any effect upon the creatinin elimination. Such results are a definite indication that the regularity of the creatinin excretion can be explained only on the basis of a similar regular formation.

While the creatinin excretion is practically constant for each healthy individual, different persons excrete different amounts, and Folin early pointed out that the chief factor determining this appeared to be the weight of the person. He further noted that the fatter the subject, the less creatinin is excreted per kilo of body weight and concluded from this that the amount of creatinin excreted depends primarily upon the mass of active protoplasmic tissue, or as Shaffer has expressed it, "Creatinin is derived from some special process in normal metabolism taking place largely, if not wholly, in the muscles, and upon the intensity of this process appears to depend the muscular efficiency of the individual." It has been found convenient to express the daily creatinin elimination in milligrams of creatinin nitrogen per kilo of body weight and this has been called the creatinin coefficient. For a strictly normal individual Shaffer has shown that this coefficient is between 7 and 11. Women eliminate less creatinin than men, and thus have slightly lower creatinin coefficients. The creatinin excretion of children is much lower than that of adults.

That the creatinin elimination is affected by different pathological conditions has been shown by numerous observations. A low creatinin elimination has been found associated with a large number of abnormal conditions, especially those accompanied by muscular weakness. Benedict and Myers observed creatinin coefficients as low as 2 in two very old decrepit women, while Levene and Kristeller found coefficients of 1.5 in several cases of muscular dystrophy in young male adults. A marked decrease in the excretion of creatinin has been observed to be associated with such conditions as exophthalmic goiter, the leucemias, diseases of the liver, especially carcinoma, muscular dystrophy, anterior poliomyelitis, certain cases of nephritis, etc. An interesting fact to note in this connection is that most of these subjects eliminate considerable amounts of creatin.

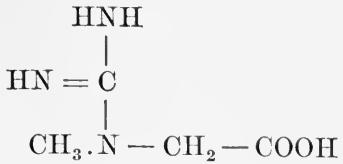
Only in the terminal stages of chronic nephritis is a decreased elimination of creatinin due to retention. Creatinin appears to be the most

readily eliminated of the three nitrogenous waste products, uric acid, urea and creatinin, and it is only in chronic nephritis or acute nephritis with partial or complete suppression of urine that retention occurs. A blood content of more than 5 mg. of creatinin to 100 c.c. has been found to be a very unfavorable prognostic sign (see preceding article, p. 441).

The excretion of creatinin has been found to be increased in fevers—typhoid, pneumonia and erysipelas. Here the rise in temperature is followed by a corresponding rise in the creatinin output. Myers and Volovic have shown that the excretion of creatinin follows closely the rise in temperature during fever, whether the hyperthermia is of infective origin or artificially induced. From this it would appear that the rise in the creatinin elimination was due entirely to the hyperthermia.

That the creatinin of the urine has its origin in the creatin of the muscle would seem obvious on *a priori* grounds, but a definite proof of this hypothesis has been beset with many difficulties. The older investigators stated that both administered creatin and creatinin reappeared in the urine as creatinin. When Folin first reinvestigated this question with accurate methods and pure creatin and creatinin, he found that 80 per cent of the administered creatinin did reappear as creatinin, but that when creatin was given in moderate amounts (1 gram to man) it not only failed to reappear as creatinin, but completely disappeared. From this Folin quite naturally concluded that creatin and creatinin were relatively independent in metabolism. In 1913 Myers and Fine(*c*) called attention to the fact that the creatin content of the muscle of a given species of animals was very constant (obviously also that of a given animal) and suggested this as a possible basis of the constancy in the daily elimination of creatinin first noted by Folin. Later they pointed out that the creatinin content of muscle was greater than that of any other tissue, and also that in autolysis experiments with muscle tissue the creatin (and any added creatin) was converted to creatinin at a constant rate of about 2 per cent daily, which is just about the normal ratio between the muscle creatin and urinary creatinin. They also found, as did Rose and Dimmitt, Lyman and Trimby, and others, that when creatin was administered to man or animals, there was a slight conversion to creatinin although a considerable percentage of the creatin reappeared in the urine unchanged if large amounts were given. These facts all go to support the view that creatinin is formed in the muscle tissue from creatin, and at a very constant rate, although no explanation of the physiological significance of this transformation can as yet be offered. Excepting possibly the kidney, the muscle normally contains more creatinin than any other body tissue and is followed by the blood which indicates that after its formation in the muscle the creatinin is carried to the kidney by the blood stream.

Creatin.—Creatin is methyl guanidin acetic acid.



It is a constant constituent of striated muscle, the concentration in man being about 0.39 per cent. The creatin content of striated muscle appears to be both constant and distinctive for a given species (see preceding article, p. 461). Creatin is also present in heart muscle in about two-thirds the concentration of striated muscle and in the testis, brain, smooth muscle and liver in much lower concentrations, the figures varying from about 0.1 per cent in the testis and brain to 0.3 per cent in the smooth muscle of the intestine and uterus, and slightly less in the liver.

Folin, in his original discussion of the subject, pointed out that although creatin is normally absent from urine, occasionally small amounts may be detected. This phase of the problem received renewed interest when F. G. Benedict(*c*) noted in starvation experiments on man that considerable quantities of creatin appeared in the urine. Following up this observation, Benedict and Myers observed the elimination of varying amounts of creatin in a large number of undernourished insane patients. Subsequent observers have shown that creatin is regularly excreted particularly in carcinoma of the liver, diabetes, muscular dystrophy, exophthalmic goiter, anterior poliomyelitis, pernicious vomiting of pregnancy, typhoid fever and pneumonia. In all except the last two conditions mentioned (fevers) this is accompanied by a lowered creatinin output, and even in fevers this is true during convalescence. Judging from the observations of Denis on the creatin content of human muscle obtained at autopsy, it would appear that the excretion of creatin was generally associated with a low muscle content. In carcinoma of the liver the creatin elimination may be very large, 1-1.5 grams.

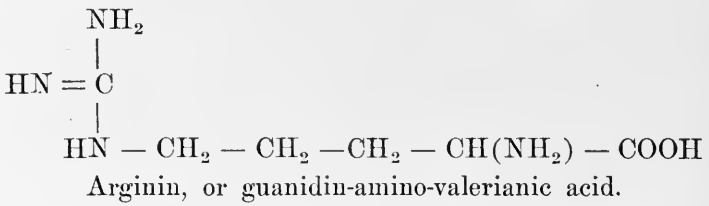
From the foregoing, it would appear that the excretion of creatin was pathological, but Rose, and also Folin and Denis(*b*), have recently observed the interesting fact that growing children excrete creatin while according to Krause normal women periodically excrete small amounts of creatin.

Muscle creatin has quite generally been regarded as the source of the urinary creatin in starvation and pathological conditions associated with undernutrition, although some workers have opposed this view. In the case of starving rabbits Myers and Fine(*d*) believed that they were able to account for the creatin lost from the muscle on the basis of urinary findings, but these observations can hardly be directly compared with pathological conditions in the human subject. McCollum and Steenbock

have shown that the pig on a high protein diet from certain sources will excrete creatin, while Benedict and Osterberg(*a*) have found that the phlorhizinized dog may eliminate very large amounts of creatin when fed on a diet of thoroughly washed meat.

Different hypotheses have been advanced to explain the excretion of creatin in children, such as under carbohydrate feeding, high protein feeding and acidosis, but the experimental evidence advanced in their support is not entirely convincing, although all these factors undoubtedly exert an influence under certain circumstances. It is now well known that the administration of carbohydrate in starvation causes a disappearance of the creatinuria. Denis and Kramer believe that the creatinuria in normal children is due to the relatively high protein intake which is the rule with practically all children, also that creatinuria may be produced in women by very high protein diets. In this view they are opposed by Rose, Dimmitt and Bartlett. Denis and Kramer further suggest that the excretion of creatin in children may also be due to the low saturation point of immature muscle owing to the low creatin content of the muscle of children and the relatively low level of protein consumption at which appreciable quantities of creatin appear in the urine. In support of this argument Gamble and Goldschmidt(*a*) have observed a practically complete elimination of ingested creatin in an infant on a high protein diet.

Granting that creatinin does come from creatin, the natural question is: What is the precursor of creatin? For this we have as yet no definite answer. On account of its guanidin group, arginin naturally suggests itself. The very close chemical relationship between arginin and creatin is apparent from the formula of arginin.



Arginin, or guanidin-amino-valerianic acid.

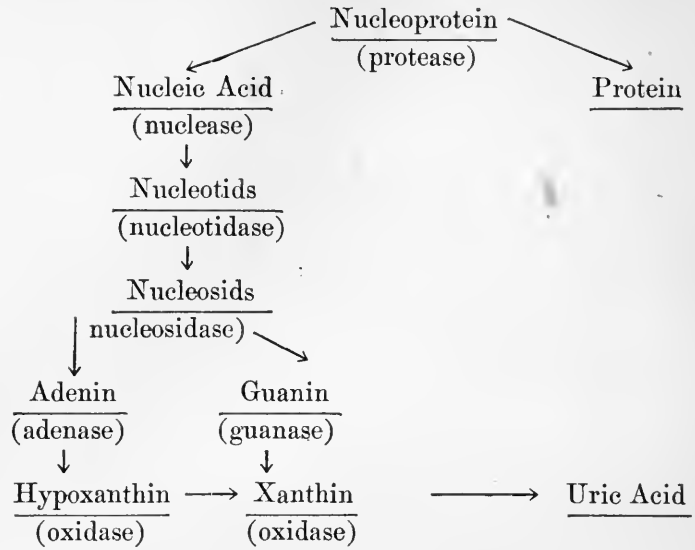
If arginin is the source it is transformed only in small part to creatin, since the amount of the daily creatinin excretion could account for only a small part of the arginin normally metabolized. From the studies of Kossel and Dakin it appears that the greater part of the arginin is hydrolyzed to ornithin and urea by the enzyme arginase, but experimental data to show that creatin is derived from arginin are inconclusive. That creatin is not present in invertebrate muscle has long been known, although the presence of arginin and likewise betain has been shown. The possibility that betain, and also the closely related cholin, are the percursors

of creatin in the vertebrate has been suggested by Riesser(*b*), who has presented evidence in experiments on rabbits suggesting that both the creatin content of the muscle and the creatinin elimination are increased after the administration of these substances. Myers and Fine(*j*) found that the creatin content of the muscle of rats was very slightly increased (2.5 per cent) as a result of feeding with edestin, a protein relatively rich in arginin. Bauman and Hines(*b*) have perfused arginin, sarcosin, methylguanidin, betain and cholin through dog muscle (hind leg) without obtaining conclusive evidence of their being creatin formers.

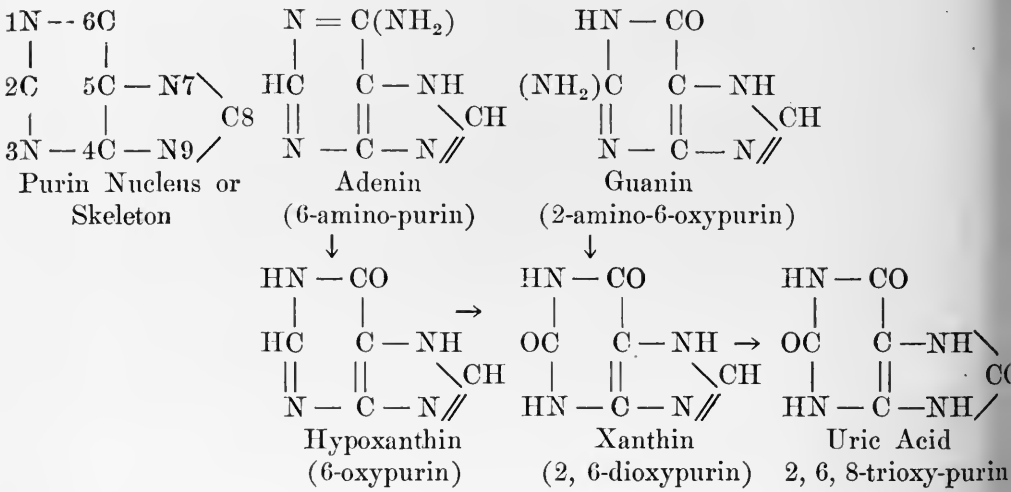
Uric Acid.—Uric acid results from the cleavage and oxidation of nucleoprotein, which is the chief constituent of all cell nuclei. Nucleoprotein is split into protein and nucleic acid. When the nucleoprotein is present in the food, this process takes place in the alimentary tract under the influence of trypsin; when the body cells are the source of the nucleoprotein this transformation takes place in the tissues probably through the agency of a similar enzyme. The protein fraction is digested in the usual way, and the nucleic acid is further transformed, ultimately yielding uric acid. Nucleic acid is a complex substance containing phosphoric acid, carbohydrate, pyrimidin and purin groups. In the molecule there is a union of 4 complex radicals called nucleotids. A nucleotid is a combination of phosphoric acid, a carbohydrate and a basic group which may be purin (*e. g.,* adenin or guanin) or a pyrimidin (*e. g.,* cytosin, uracil or thymin). In nucleic acids of plant origin, the carbohydrate is usually a pentose (d-ribose), while a hexose is the carbohydrate found in animal nucleic acids. Animal nucleic acids further differ from the plant variety in having the pyrimidin, thymin, instead of uracil.

The nucleic acid is split into its component nucleotids, which experience another cleavage resulting in the liberation of phosphoric acid, leaving carbohydrate-purin and carbohydrate-pyrimidin combinations. The latter compounds are known as nucleosids and are eventually split, liberating the free purin and pyrimidin bases. The purin bases, adenin and guanin, are then converted respectively into hypoxanthin and xanthin, this change being accomplished by the enzymes adenase and guanase. Finally by means of an oxidizing enzyme, xanthin is transformed to uric acid. This process is graphically represented on the following page, the enzymes being enclosed in parenthesis.

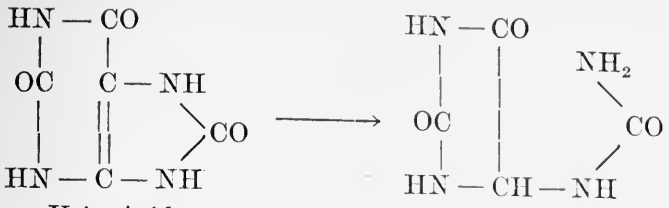
The pyrimidins, especially cytosin, have been suggested as possible purin precursors by Kossel, but no experimental evidence has been adduced in support of this hypothesis. The fate of the pyrimidins appears to be quite uncertain. Mendel and Myers found that when the three pyrimidins found in nucleic acid were administered to man or animals they reappeared in the urine unchanged, and Wilson has made similar observations regarding the pyrimidin nucleosids.



We are familiar with the chemical structure of the purins owing chiefly to the researches of Emil Fischer and his pupils. An appreciation of the chemical structure of this group of compounds is of material aid in obtaining an adequate understanding of purin metabolism.



It has been claimed that in man about half the uric acid is subject to a further enzymatic change (uricolysis). This, however, is still a disputed question although in animals the greater part of the uric acid is undoubtedly converted to allantoin.



Uric Acid Allantoin

The difference in the fate of uric acid in man, on the one hand, and in the dog, rabbit, etc., on the other, is probably a quantitative one. Qualitatively there is no dissimilarity, for traces of allantoin do appear in human urine, and the urines of the lower animals do contain small amounts of purins (Hunter and Givens(*c*)). It is especially significant from the standpoint of comparative physiology to learn that as far as their purin metabolism is concerned, the monkey ranks with the lower animals rather than with man. The purin metabolism of man, then, is unique in that uric acid represents the principal excretory product. It is of further interest to note that human blood contains from 10 to 60 times as much uric acid as the blood of the rabbit, cat and sheep. Whereas the blood of these animals contains from 0.05 to 0.2 mg. of (free) uric acid per 100 c.c. of blood, normal human blood contains 2 to 3 mg. A similar difference has been found in the tissues of man and animals (Fine). This furnishes additional evidence pointing to the relative indestructibility of uric acid in man.

From the fact that in birds the end product of nitrogenous metabolism in general is uric acid, apparently of synthetic origin, the attempt has been made to demonstrate a similar formation in man, but without conspicuous success. For the present, uric acid must be regarded as arising solely from the oxidative transformations of the purin bases, whether they already exist in the body or have been introduced from without.

The precursors of uric acid, nucleoprotein and purin bases, may be present in the food or disintegrating cellular material of the body. In the former case, the uric acid is said to be of "exogenous origin," in the latter, of "endogenous origin." The output of endogenous uric acid will be determined by the extent of the body cell activity. During starvation, for example, the 24 hr. uric acid elimination may vary from 0.1 to 0.2 gram, which may be increased to 0.2 to 0.4 gram on a purin-free diet. This diet contains no uric acid precursors, and could, therefore, cause the increased uric acid output only indirectly. It is quite generally accepted that the augmented output of uric acid following the ingestion of a purin-free diet is due to the necessarily increased activity of the digestive glands, thus raising the level of endogenous purin metabolism (Mareš, Mendel and Stehle). The administration of drugs, such as pilocarpin, which stimulates glandular activity, also increases the uric acid output, while atrophin,

a glandular depressant, causes a reduction. With uric acid yielding foods as meat, meat extracts, pancreas, liver, thymus, peas, beans, etc., the output will, of course, be the sum of endogenous and exogenous uric acid. Mendel and Wardell have demonstrated that uric acid excretion may be very definitely increased by the taking of methylated xanthins in coffee, tea and cocoa, obviously indicating a demethylation of these purins. On a mixed diet 0.5 to 0.6 gram of uric acid may be taken as the average output of the human adult.

The greatest increase in uric acid elimination is observed in leucemia, as much as 12 grams having been found to be excreted in 24 hours. This high elimination of uric acid is without doubt to be referred to the enormous increase in the number of leucocytes and consequent leucolysis. An increased uric acid excretion is observed in other diseases associated with a high grade of leucocytosis. Although in gout deposits of sodium urate may be found in certain of the articular cartilages, and the blood uric acid increased owing to faulty elimination, still the quantitative excretion of uric acid in gouty individuals does not differ markedly from that found normally. It may, however, be noted that for two or three days preceding an attack of acute gout the uric acid elimination is diminished; while during and for a few days after the attack it may maintain a level somewhat above normal.

It has been recognized for some time that the excretion of uric acid was stimulated by the administration of salicylic acid and phenylcinchoninic acid and their derivatives, and they have frequently been referred to as "uric acid eliminants." Myers and Killian(*c*) have recently pointed out, however, that this action is not specific for uric acid. It has been found that in suitably selected cases, having slightly increased blood urea (and possibly also creatinin) findings, administration of the above compounds will lower the blood concentration of these constituents as well as the uric acid.

Ordinarily uric acid is present in the urine in combination with sodium, potassium or ammonium. Only when the urine is especially acid does uric acid itself separate out. When the urine is concentrated or after the ingestion of considerable meat, pancreas, etc., urates may be deposited shortly after the urine is voided. In other cases such deposits may form on standing in a cool place.

Purin Bases.—A small portion of the purin bases, adenin, guanin, hypoxanthin and xanthin escape conversion to uric acid, and appear unchanged in the urine. About 0.02 to 0.05 gram of such compounds may be eliminated.

Hippuric Acid.—Hippuric acid is a combination of glycocoll and benzoic acid. By this conjugation which takes place in the kidney, although it may be formed elsewhere (Kingsbury and Bell), the body is able to defend itself against the more toxic benzoic acid. For this reason small

amounts of benzoic acid or sodium benzoate would appear to be harmless. Hippuric acid is found in the urine of herbivorous animals, such as the horse and cow, in large amount, but only about 0.7 gram per day occurs in human urine. Certain fruits and berries, cranberries in particular, contain appreciable amounts of benzoic acid, while certain aromatic substances of vegetables are ultimately converted to benzoic acid. It may also be formed by the putrefactive decomposition of the phenylamino acids in the intestine. Benzoic acid or sodium benzoate is often used as a preservative in canned fruit and catsup. All these factors contribute to the hippuric acid output. It is stated that hippuric acid is decreased in fevers and in certain kidney disorders where the synthetic activity of the renal cells is diminished.

Oxalic Acid.—Oxalic acid in the form of calcium oxalate usually occurs in the urine in very small amounts, about 0.02 gram in 24 hrs. Oxalic acid is probably formed from the metabolism of proteins and fat. Its output may be increased by the ingestion of foods which contain oxalic acid. Such foods are cabbage, spinach, apples, grapes, etc.

Aromatic Oxyacids and Derivatives.—Under this heading may be mentioned phenol, *p*-cresol, indoxyl, scatoxyl, indol acetic acid and homogentisic acid. These substances are all formed from the amino-acids, tryptophan, tyrosin and phenylalanin. Homogentisic acid is apparently formed as a result of abnormal oxidation of the last two amino-acids mentioned. It occurs in alkaptonuria, a comparatively rare anomaly of metabolism. In this condition the excretion may amount to as much as 16 grams per day, although ordinarily it is less, i. e., 3 to 5 grams. Intestinal putrefaction (in rare instances, putrefaction elsewhere in the body) gives rise to the formation of the other bodies mentioned. Phenol, *p*-cresol, and indoxyl are eliminated in the urine partly in combination with sulphuric acid, constituting the ethereal sulphates. Indoxyl-potassium-sulphate, or indican, appears to depend upon the amount of intestinal putrefaction, and to be an excellent index of it, but the same can hardly be said of the ethereal sulphates as a whole, indicating that in part they have another origin. Under normal conditions from 5 to 20 mg. of indican are excreted per day, but in conditions showing excessive intestinal putrefaction as much as 200 mg. may be eliminated. In certain of these cases indol acetic acid is excreted, giving rise to the so-called urorosein reaction. According to the recent studies of Folin and Denis the larger part of the phenols (phenol, *p*-cresol, etc.) are excreted in the free form. The daily elimination of phenols appears to average about 300 mg., of which about 60 per cent is free and 40 per cent conjugated.

Sugar.—Sugar appears to be present in normal urine in very small amounts. As a result of the recent studies of Benedict, this subject has attracted considerable interest. Normal urine apparently contains from 0.02 to 0.2 per cent of sugar with an average of about 0.07 per cent. Of

this sugar roughly half is fermentable. The 24 hr. elimination may vary from 0.5 to 1.5 grams, but from a large series of analyses made by Croll on hospital cases the daily average would appear to be about 0.7 gram. Unless the carbohydrate tolerance is definitely disturbed, larger amounts do not appear to be excreted. Even in hyperthyroidism comparatively normal values are found.

Inorganic Constituents

The inorganic constituents of the urine are chiefly the sodium, potassium, calcium, magnesium and ammonium salts of hydrochloric, phosphoric and sulphuric acids. The salts of sodium and potassium are eliminated almost exclusively in the urine, but, as pointed out in the section on feces, much more calcium and magnesium are eliminated by the intestine than by the kidneys, these elements being largely in combination with phosphoric acid. The average inorganic solid elimination in the urine amounts to about 20 grams daily, sodium chlorid ordinarily contributing considerably more than half of the total. The average elimination of these different constituents for the human adult may be given as follows:

	Grams
Sodium as Na_2O	6.0
Potassium as K_2O	3.0
Calcium as CaO	0.3
Magnesium as MgO	0.2
Ammonium as NH_3	0.6
Iron as Fe	0.003
Chlorids as Cl	7.0
Phosphates as P_2O_5	2.5
Sulphates as SO_3	2.0

Long and Gephart have made fairly complete mineral analyses on the composite urines of six healthy adults. They found that they could obtain an almost exact balance between acids and bases, if they assumed that four-fifths of the phosphoric acid was held as dihydrogen phosphate and one-fifth as monohydrogen phosphate. On this basis they suggested the arbitrary salt combinations given in tabular form on the next page.

Chlorids.—The amount of chlorids, chiefly sodium chlorid, excreted per day is dependent upon the food chlorids. The elimination is quite variable but ordinarily falls between 10 and 15 grams. Some people ingest very large amounts of salt with their food. This salt is absorbed and passes rapidly through the kidneys into the urine. In starvation the sodium chlorid excretion is reduced to a minimum. The same conditions

	Grams
Sodium chlorid	13.00
Potassium chlorid	4.23
Calcium sulphate	0.52
Magnesium sulphate	0.61
Ammonium sulphate	1.52
Ammonium urate	0.58
Potassium urate	0.03
Potassium phenyl sulphate	0.42
Potassium dihydrogen phosphate	2.56
Potassium monohydrogen phosphate	0.86

obtain in cases of carcinoma of the stomach, resulting in stenosis of the pylorus, essentially a condition of starvation. The sodium chlorid elimination is decreased by those conditions which favor its removal from the blood through other channels, *e. g.*, cases of diarrhea, rapidly formed transudates and exudates, such as pleurisy with effusion. It may be pointed out that for several days after the reabsorption of an exudate, the chlorid excretion may be greatly increased, and is here a favorable diagnostic sign. Diminished chlorid elimination is observed during the crises of acute febrile diseases, especially pneumonia and in nephritis with edema, in the latter case because of the relative impermeability of the kidney to salts. In febrile diseases it is worthy of note that the elimination of chlorids progressively decreases as the febrile process approaches its crisis, and tends to rise to its original level during convalescence. It has been observed that in pneumonia there is, if anything, a decreased chlorid content of the blood, while in exceptional cases of nephritis with marked edema, the chlorids of the whole blood may rise from the normal of 0.45-0.50 per cent to as high as 0.7 per cent. Such cases do not generally show marked nitrogen retention.

Phosphates.—Two types of phosphates are present in urine, the *alkaline phosphates,* salts of the alkali metals, and *earthy phosphates,* salts of the alkaline earth metals. In the normally acid urine the larger part of the phosphoric acid is generally present as Na or KH_2PO_4, the dihydrogen phosphate. The urinary excretion of phosphates as P_2O_5 amounts to 1 to 5 grams, with an average of 2.5 grams. This originates to a small extent in the setting free of phosphoric acid in protein metabolism, but to a greater extent in the phosphates of the foods. The extent to which the latter control the phosphate excretion in the urine depends upon the relative abundance of alkali and alkali-earth phosphates. The alkali-earth phosphates are difficultly absorbable and hence are in great part eliminated directly through the feces, thus contributing but little to urinary phosphate. Ordinarily about two-thirds of the phosphorus is eliminated in the urine, but a diet containing a very large amount of

milk, for example, will increase the fecal excretion. The alkali phosphates are absorbed and add to urinary phosphate to a large extent, but even these may be converted into alkali-earth phosphates in the body and be in part excreted into the intestine, reappearing in the feces. About 1 to 4 per cent of the phosphorus excreted is in an organic combination of unknown nature. The phosphate elimination is said to be increased in periostosis, osteomalacia, rickets and after copious water drinking; and decreased in acute infectious diseases, pregnancy and diseases of the kidney. Sherman and Pappenheimer have recently shown that phosphorus may be made the limiting factor in experimental rickets in rats, while a number of investigators have observed a retention of inorganic phosphorus in the blood in nephritis. The retention of acid phosphate, or rather the inability to excrete acid phosphate, is probably a very important factor in the latter condition. At times a turbidity due to phosphates may be observed. This is sometimes erroneously interpreted as indicating an increased elimination of phosphates, "phosphaturia." It is more likely due to a condition of decreased acidity and is more properly termed "alkalinuria." This precipitation of phosphates may also be due to an unusual amount of calcium which would form one of the less soluble phosphate combinations.

Sulphates.—Sulphur is excreted in three forms: *oxidized* or inorganic sulphur, *e. g.,* the sulphates of sodium, potassium, calcium and magnesium; *ethereal sulphur, e. g.,* sulphates of phenol, indoxyl, scatoxyl, cresol, etc.; *neutral sulphur, e. g.,* cystin, cystein, taurin, hydrogen sulphide, etc. The greater part of the sulphur of the urine is present in the oxidized or inorganic form, averaging rather more than 2.0 grams calculated as SO_3, this as a rule being about 10 times the amount of ethereal sulphates excreted. The ethereal sulphates normally amount to 0.20 gram and the neutral sulphur to about the same amount, although sometimes being more and sometimes less. The neutral sulphur elimination is relatively uninfluenced by the diet, and Folin regards it as being analogous to the creatinin. An idea of the distribution of the sulphur on a high and on a low protein diet may be obtained from the table on page 486. The inorganic sulphur of the urine arises mainly from the oxidation of the sulphur of the protein, and is thus increased by those conditions which stimulate protein metabolism such as acute febrile diseases, and decreased when the rate of metabolism is lowered. The ethereal sulphates of the urine are increased by excessive formation and absorption from the intestine of products of putrefaction, *e. g.,* phenol, indol, skatol, or by the administration of similar aromatic bodies such as phenol, cresol, resorcinol, etc.

Sodium and Potassium.—The quantity of sodium ordinarily present in the urine parallels quite closely the amount of chlorin. The excretion in the healthy adult may be given as 4 to 8 grams with an average of about 6 grams calculated as Na_2O. The proportion of Na to K is fairly

constantly maintained at about 5:3. It is well known that foods rich in potassium, such as meat and potatoes, require more salt than other foods. The quantity of both of these elements excreted depends chiefly upon the food. In starvation or during fever the potassium of the urine may be in excess owing to a destruction of the body's own tissues.

Calcium and Magnesium.—Since the larger part of the calcium and magnesium eliminated are excreted in the feces it is always necessary to have data on the fecal excretion of these elements to make satisfactory deductions (see discussion on page 511). Under different conditions of diet the calcium excretion in the urine may vary from 0.1 to 0.5 gram calculated as CaO, and the magnesium from 0.1 to 0.3 gram calculated as MgO depending upon the diet; sometimes the calcium is in excess in the urine and sometimes the magnesium. In a series of 25 healthy adults Nelson and Burns found the calcium in excess in 17 and the magnesium in 8. The figures for the CaO ranged from 0.13 to 0.49 gram and for the MgO from 0.12 to 0.30. In this connection they state that either calcium or magnesium may be excreted by way of the urine in the larger amount, in the normal individual. Whichever element predominates does so constantly, or very nearly so, and seems to be independent of the character of the food ingested. The excretion of calcium and magnesium does not necessarily run parallel pathologically, since there may be a retention of magnesium in certain bone disorders accompanied by a loss of calcium; for example, osteomalacia. Very little is known, however, about the pathological excretion of these elements. The lime salts absorbed are in great part excreted again into the intestine, and the quantity in the urine is therefore no measure of their absorption. The introduction of readily soluble lime salts or the addition of hydrochloric acid to the food may therefore cause an increase in the quantity of lime in the urine, while the reverse takes place on the addition of alkali phosphate to the food. In other words, the balance between the acid- and base-forming elements in the foods has a very important bearing upon the excretory path of these elements and phosphorus.

Iron.—Iron exists in the urine only in very small amount (1 to 5 mg. per day) and that in organic form. It is largely eliminated by the intestine.

Feces

It has long been the common notion that feces are composed of the residues of undigested food. In health, however, this is far from the truth. It is easy to comprehend that the nitrogenous waste products of the urine are derived from the catabolism of protein in the body, but since the intestinal canal is a long tube open at both ends through which undigested material may pass, it has been difficult to appreciate that

under normal conditions the feces are composed largely of intestinal secretions and excretions, together with bacteria, cellular material from the intestinal walls and food residues. Furthermore as Mendel(*a*) and his coworkers have shown, the feces is the normal path for the elimination of a number of foreign inorganic elements, such as strontium, barium, etc. As a proof that feces are a true secretion, it has been shown by F. Voit that the material secreted in an isolated loop of the intestine of a dog is of similar composition, and contains the same amount of nitrogen as the feces of the normal intestine through which food is passing. Especially significant are the observations of Mosenthal(*a*), who also worked with isolated intestinal loops, and estimated that the succus entericus contained nitrogen equivalent to 35 per cent of the nitrogen ingested, and 300 to 400 per cent of the nitrogen of the feces. Nitrogen equivalent to at least 25 per cent of that of the intake must therefore have been reabsorbed. Prausnitz has pointed out that the nitrogen content of the feces of the same individual on a meat and on a rice diet are practically identical, indicating the metabolic origin of the nitrogen. He defines normal feces as those resulting from the eating of any food that is completely digested and absorbed. Such foods as milk, cheese, rice, eggs, meat, macaroni and white bread are largely available for the use of the organism and consequently yield a comparatively small amount of feces. On the other hand, the cellulose containing vegetables do not possess this availability and therefore yield a much more copious fecal output. Cabbage is an excellent illustration of such a vegetable. It is logical to expect that on a diet whose constituents are not entirely available, not only would the amount of feces be increased by the undigested cellulose, but also the nitrogen content would be increased because of the large amount of digestive juices secreted, the large volume of food and the accompanying increased peristalsis. Although the exact composition of a large part of the organic material eliminated in the feces is unknown, still it is now recognized that bacterial substance forms a considerable part of this material.

The fact that about one-third of the dry matter of normal human feces consists of bacteria, and at least one-half of the nitrogen of the feces is bacterial in its origin, serves to emphasize the importance of bacteria in the intestinal canal, though experimental evidence would indicate that the presence of this large number of bacteria is a normal and even useful condition. MacNeal, Latzer and Kerr, who have devoted considerable attention to the bacterial content of the feces, find that in normal subjects the bacterial dry substance varies between 1.8 and 9.2 grams with an average of 5.3 grams per day, while the bacterial nitrogen ranges between 0.2 and 1.0 gram with an average of 0.6 gram, this latter figure constituting 46.3 per cent of the fecal nitrogen. Of the fecal bacteria they find that 80.7 per cent are Gram negative (45.9 per cent *B. coli* type), 17.0

per cent Gram positive and 2.3 per cent free spores. Mattill and Hawk(*a*), who employed the MacNeal method slightly modified (no ether extraction used), obtained slightly higher results on two subjects who were followed for several weeks. They found that the bacterial nitrogen averaged 53.9 per cent of the fecal nitrogen and the bacterial dry substance 8.27 grams. Under normal conditions the bacteria probably derive their sustenance in considerable part from the intestinal secretions and excretions, but pathologically they may decompose appreciable amounts of partially digested protein and carbohydrate.

In nurslings the bacterial flora is relatively simple, though later in life the number of these bacterial forms becomes very large. The dominant organism in nurslings is *B. bifidus* (*B. acidophilus* of Moro is also present), but this is ultimately replaced by *B. coli* and *B. lactis aërogenes.* Other organisms which may be observed are *coccal forms, B. welchii,* and in certain cases, *B. putrificus* (Herter(*d*)). These last two organisms Herter is inclined to associate with conditions of excessive putrefaction in the intestines. MacNeal has pointed out, however, that *B. welchii* can generally be detected in normal stools. In early life the products of intestinal decomposition are very small in amount, and, as would be expected, the number of putrefactive bacteria are few. One finds, however, in middle life a large number of persons in whom the putrefactive conditions in the intestine are distinctly more active than was the case earlier in life. Apparently the most important factors in bringing about this strongly proteolyzing type of bacterial flora are the consumption of an overabundance of protein food, combined with inadequacy in the digestive juices, delayed absorption, and insufficient motility in the alimentary canal. Very little decomposition takes place in the large intestines under the action of *B. coli,* however, if the absorption in the small intestine has been good. Rettger and his coworkers have recently pointed out that the daily administration of 150-300 grams of lactose or dextrin to adults will, with few exceptions, bring about a marked change in the bacterial flora in which the usual mixed types of bacteria give way to *B. acidophilus,* which is a normal intestinal organism, but which is present in the intestine after early infancy in relatively small numbers only. This method would appear to possess interesting possibilities of therapeutic usefulness.

Amount.—Upon the ordinary mixed diet, the daily fecal excretion of the adult male averages from 100 to 150 grams, with a solid content varying between 20 and 40 grams. Upon a vegetable diet the fecal output will be much greater, reaching 350 grams with a solid content of 75 grams, and even more. This being the case, data on variations in the daily excretion are of little practical significance, except where the composition of the diet is accurately known. Lesions of the digestive tract, a defective absorptive function, or increased peristalsis, as well as admix-

ture of mucus, pus, blood and pathological products of the intestinal wall may cause the total amount of feces to be markedly increased.

Consistency.—The form and consistency of the feces is dependent, in large measure, upon the nature of the diet. Under normal conditions the consistency may vary from a thin, pasty composition to a firmly formed stool. Feces which are exceedingly thin and watery generally have a pathological significance.

Color.—The fecal pigment of the normal adult is hydrobilirubin, also called stercobilin. It has its origin in the bilirubin of the bile, being formed by the reducing action of certain bacteria. Hydrobilirubin is probably identical with the urobilin of the urine. This pigment is present in both the urine and feces, partly in the form of its chromogen, urobilinogen. This is transformed to urobilin under the action of light. Normally hydrobilirubin appears to be largely reabsorbed and converted to bilirubin. In pernicious anemia the destruction of red cells is so rapid that it cannot be reabsorbed, thus leading to a marked excretion of the reduced pigment in the stool, a very valuable point in the differential diagnosis of primary and secondary anemia. (It is not increased in secondary anemia.) In certain liver diseases there is sometimes a breakdown in the ability to reconvert urobilin to bilirubin, which leads to the appearance of the pigment in the urine in abnormal amounts. Neither bilirubin nor biliverdin occur normally in the feces of adults, although bilirubin sometimes occurs in the stools of nursing infants.

The diet is the most important factor in determining the color of the feces. On a mixed diet the stools may vary in color from light to dark brown, on an exclusive meat diet the stools are brownish black, while on a milk diet they are invariably light colored. Cocoa produces reddish brown feces, while with certain berries the feces may be almost black. Pathologically, absence of bile, or any condition producing a large amount of fat, gives clay colored stools; blood from the upper part of the alimentary tract yields "tar feces."

Odor.—The odor of normal feces is generally stated to be due to skatol and indol. However, these aromatic putrefactive substances are generally found in such small amounts as to be an insufficient explanation on this point. Hydrogen sulphid and methylmercaptan probably play a certain part in the disagreeable character of the odor. The intensity of the odor depends to a large extent upon the diet, being very marked in stools from a meat diet, much less marked in stools from a vegetable diet, and often hardly detectable on stools from a milk diet. The stool of the infant is ordinarily quite odorless, and any decided odor may generally be traced to some pathological source.

A simple division of fecal material may be based upon the separation afforded by the customary procedures, viz., the estimation of the total nitrogen, ethereal extract, carbohydrate residues and ash. The results

obtained with these methods have yielded data of great scientific importance, though the time required and the nature of the results render them of comparatively little value diagnostically.

An idea of the approximate composition of feces in the normal human adult may be obtained from the tabular data below. Except for the moisture content, the percentage figures are on a dry basis.

	Grams	Per Cent
Moist feces	120	..
Air dry feces	30	..
Moisture content	..	75
Nitrogen	1.8	6
Ether extract	6.0	20
Carbohydrate	1.0	3
Ash	4.5	15

Nitrogenous Substances.—Three sources are usually considered as contributing to the nitrogenous material excreted in the feces; food residues, residues of the digestive juices and cellular material from the intestinal wall, and bacteria and their products. The quantity of this nitrogen normally amounts to from one to two grams and from four to eight per cent of the dry feces. As already pointed out 0.5 to 0.8 gram of nitrogen is daily eliminated in the form of bacteria. This constitutes just about half of the fecal nitrogen and corresponds almost exactly with what is ordinarily spoken of as the "metabolic nitrogen." Upon a meat diet the food residues represent almost nothing under normal conditions, i. e., the muscle protein is practically 100 per cent utilized, and furthermore the fecal nitrogen is almost wholly "metabolic" in origin. In the case of vegetable proteins it has been a matter of common observation that the utilization was not so good as with animal proteins. This in part at least is explained by the inaccessibility of certain of the vegetable proteins to the digestive juices, for as Mendel and Fine have shown, the proteins of the wheat, and probably also of the barley and corn, are as well utilized as meat, when taken in pure form or freed from extraneous cellular substance. With legumes the utilization does not appear to be quite so good. In order to calculate the digestibility of various proteins and make allowance for the "metabolic nitrogen" Mendel and Fine propose the determination of the volume and nitrogen of feces resulting from the material under investigation, with the subsequent determination of the fecal nitrogen resulting from a nitrogen-free diet to which has been added an amount of indigestible non-nitrogenous matter that will yield approximately the same volume of feces as in the first instance. The excess of fecal nitrogen of the first test over the second is presumably due to the undigested or unabsorbed nitrogenous matter of the food material.

With regard to the elimination of fecal nitrogen under pathological conditions, observations show that it is increased in biliary obstruction, intestinal fermentative dyspepsia, and diarrhea; and decreased in chronic constipation.

A great variety of substances may be formed by bacterial action upon protein or its cleavage products. Among such may be mentioned indol, skatol, phenol, indol acetic acid, various oxyacids, in certain instances, putrescin and cadaverin, etc. That intoxication may result from poisonous products formed by bacterial action can hardly be questioned, though just what the substances are that exert this action cannot be stated at the present time. Much attention has been devoted to the products of bacterial action on tryptophan, viz., indol acetic acid (urorosein of urine), skatol and indol. Myers and Fine found comparatively large amounts of skatol and indol in the stools of pellagra patients. In many of the patients the stools were rather soft. Ordinarily skatol appears to be observed in the feces much less frequently than indol, but the reverse was true in these cases. In the case showing the most severe putrefaction, the skatol of the feces averaged 51 mg. and the indol 21 mg. per day. The indican of the urine was much lower in this case than in several other subjects who excreted much smaller amounts of skatol and indol in the feces. It seems questionable whether the skatol and indol in the amounts absorbed in this way have any toxic properties. The presence of large amounts of indican in the urine, however, is excellent evidence of increased intestinal putrefaction.

Ethereal Extract.—The bodies which go to make up this ethereal extract are the neutral fats, free fatty acids (and fatty acids in the form of soaps when an acidified solvent has been employed), and coprosterol (stercorin of Flint) formed from cholesterol by the action of reducing bacteria. Myers and Wardell found the coprosterol (and cholesterol) of dry feces to vary between 0.5 and 1.5 per cent, the high figures being found in soft stools. The ethereal extract ordinarily forms from 12 to 25 per cent of the dry weight of the feces. The utilization of fat varies under normal conditions from 90 to 95 per cent, depending upon the source of food. The higher fats such as stearin are much less readily assimilated. In biliary obstruction as much as 70 grams of fat may be eliminated in the feces, forming 50 per cent of the dry weight of the material. In various conditions associated with defective fat digestion (pancreatic disease) or defective fat absorption increased amounts may be eliminated, while in chronic constipation the amount may be decreased. In both biliary obstruction and pancreatic disease the fat utilization has been found to be as low as 25 per cent.

Carbohydrate Residues.—Normally feces may yield on hydrolysis reducing substances equivalent to from one-half to two grams of glucose or from two to six per cent of the dry weight of the feces. Although the

utilization of carbohydrate has generally been given as about 98 per cent, it is evident from these figures that on a diet of 300 to 400 grams carbohydrate it is above 99 per cent. As Langworthy and Deuel have recently pointed out, contrary to the general assumption, even raw starch may be quite well utilized. Ordinarily starch digestion does not seem to be interfered with, though the amount of carbohydrate eliminated in the severer catarrhal conditions of the intestine may be slightly increased. One question to be asked with regard to all carbohydrate material is, are the enzymes of the alimentary canal capable of hydrolyzing it? As Mendel and certain of his pupils have pointed out, there appear to be no enzymes in the digestive tract capable of attacking certain of the more complex carbohydrates, such as agar agar, Iceland moss, inulin, certain galactans, etc.

Ash.—The inorganic constituents of the feces are derived partly from the intestinal secretions and partly from the food. The proportion which comes from the food varies with the nature of the diet. A purely meat diet results in a lowering of the ash content of the feces, while with a milk diet the ash is increased, owing to the presence of unabsorbed lime. On an ordinary mixed diet the ash of the feces generally falls between 10 and 15 per cent of the dry weight, but on a milk diet values of 25 to 35 per cent are found, about 40 per cent of which is due to calcium. Pathologically, Cammidge has occasionally observed cases of chronic colitis in which as much as 45 to 50 per cent of the dry weight of the feces consisted of inorganic ash.

A general idea of the composition of human feces may be obtained from the table on the next page taken from Myers and Fine, giving the fecal analyses of a series of pellagra patients. Except for Case 5 (a male) the patients were all rather small women. It is not believed that the findings differ very materially from what would be found in other hospital cases on similar diets, and with similar fecal movements. The cases have been divided into two groups, the first group having well formed stools, and the second group soft or diarrheal stools. The diet in all cases was lactovegetarian, which probably explains the rather high ash figures obtained. Estimations of iron and sodium were not made. The figures recorded in the literature for the daily excretion of sodium (as Na_2O) in the feces amount to 0.25 to 0.35 gram, and for iron (as FeO) to 25 to 40 mg. (The daily excretion of iron in the urine varies from 1 to 5 mg.) An idea of the comparative importance of the intestines and kidneys as paths for the elimination of various elements may be obtained from the table on page 511. The figures are computed from the previous table and urinary data for the same period.

An inspection of the table shows that in the first group of cases the total nitrogen and total sulphur parallel each other very closely, as probably might be expected from their common origin (protein). With diarrhea, sulphur does not appear to be quite as well absorbed as the nitrogen.

AVERAGE DAILY COMPOSITION OF FECES IN PELLAGRA

Case	Weight, Air Dry Grams	Moisture Content Per Cent	Nitrogen Per Cent	Ether Extract		Carbohydrate		Ash		Daily Average in Grams							
				Grams	Per Cent	Grams	Per Cent	Grams	Per Cent	H_2O	N	SO_3	NaCl	P_2O_5	CaO	MgO	K_2O
1	23.6	74	4.8	2.7	11	0.9	3.6	4.95	21.0	66	1.13	0.18	0.18	1.01	2.00	0.31	0.87
2	19.0	74	4.0	4.5	24	0.8	4.1	4.52	23.8	54	0.75	0.14	0.23	1.44	1.75	0.34	0.76
3	13.7	76	3.7	3.2	23	0.4	2.8	3.06	22.3	44	0.51	0.15	0.10	0.71	1.26	0.18	0.23
4	24.4	78	6.1	4.5	18	0.6	2.5	5.05	20.7	89	1.49	0.31	0.63	1.39	1.94	0.33	0.76
5	21.6	79	4.7	6.9	32	0.6	2.6	4.49	20.8	81	1.01	0.19	0.38	1.08	1.40	0.25	0.53
Av.	20.4	76	4.7	4.4	22	0.7	3.1	4.41	21.7	67	0.96	0.19	0.30	1.13	1.67	0.28	0.73
6	28.6	79	6.4	6.4	22	1.1	4.1	4.49	15.7	119	1.84	0.38	0.71	1.16	1.70	0.20	1.30
7	27.4	81	5.1	5.3	19	1.2	4.5	5.84	21.3	120	1.41	0.30	0.68	1.03	2.65	0.43	1.41
8	23.1	82	6.5	4.4	19	0.9	4.0	3.74	16.2	106	1.49	0.38	0.55	0.97	1.49	0.25	0.44
9	9.7	84	5.5	2.1	22	0.2	2.6	2.16	22.3	49	0.54	0.13	0.25	0.62	0.80	0.12	0.54
10	36.4	84	6.5	2.9	8	3.3	9.0	5.68	15.6	197	2.35	0.57	0.85	1.17	2.17	0.35	0.97
11	31.0	85	4.8	10.9	35	1.2	3.9	5.86	18.9	169	1.47	0.38	0.42	1.49	1.99	0.33	1.52
12	22.0	88	6.3	3.2	15	1.7	7.6	3.96	18.0	160	1.36	0.30	0.70	0.76	1.08	0.26	0.76
13	22.8	88	6.0	3.3	14	1.3	5.7	4.33	19.0	172	1.10	0.42	0.67	0.88	1.44	0.30	0.98
14	41.1	89	5.6	10.5	25	1.4	3.4	7.15	17.4	326	2.28	0.63	1.11	1.60	2.40	0.40	2.00
Av.	26.9	84	5.9	5.4	20	1.4	5.0	4.80	18.3	158	1.54	0.39	0.66	1.07	1.75	0.29	1.10

COMPARATIVE IMPORTANCE OF THE INTESTINE AND KIDNEYS AS EXCRETORY CHANNELS

Case	Percentage Output of Material Eliminated in Feces of Total Output of Both Urine and Feces							
	H_2O	N	S	Cl	P	Ca	Mg	K
1. M. F. (b)	7	13	9	3	33	90	76	23
2. M. L.	4	7	7	2	43	92	83	18
3. M. F. (a)	6	6	10	2	28	89	69	9
4. C. T.	6	14	15	4	40	88	66	18
5. J. A.	8	10	10	6	35	89	65	20
Averages	6	10	10	3	36	90	72	18
6. E. C.	7	18	19	8	35	80	46	28
7. A. N.	6	10	10	7	23	90	59	28
8. M. T.	7	12	15	5	27	85	54	11
9. M. McH. (a)	14	8	12	13	31	85	60	34
10. R. N.	20	22	29	9	35	94	74	24
11. L. G.	13	14	18	4	44	93	81	30
12. M. McH. (b)	33	21	26	18	33	92	84	29
13. M. S.	17	11	15	7	30	81	77	24
14. B. B.	32	21	26	16	42	92	77	38
Averages	16	15	19	9	33	89	68	27

Although normally very little chlorid is eliminated by the intestine, the amount found in the stools may be considerably increased in diarrhea. About one-third of the total phosphorus output of the intestine and kidneys is found in the stools. The percentage output in the feces of both calcium and magnesium is high, due probably to the lactovegetarian diet, which resulted in a poor absorption of compounds of these elements. On a mixed diet about 60 per cent of both calcium and magnesium are ordinarily eliminated in the feces of adults, although on milk diets the stools of infants may contain considerably more than 90 per cent of these elements. As might be anticipated from our knowledge of potassium salts, a very appreciable amount of this element is eliminated in the feces, and diarrhea considerably accentuates this elimination. Although diarrhea very definitely reduces the absorption of nitrogen, sulphur, chlorin and potassium, it appears to be almost without influence on the phosphorus, calcium and magnesium.

It is evident, therefore, that calcium, magnesium and iron are normally eliminated chiefly by the intestine. Failure of absorption is partially responsible for this, but in part these elements are secreted into the intestines, as are such similar elements as strontium and barium (Mendel). The elimination of calcium and phosphorus are interrelated both as to total excretion and path of elimination. An increased ingestion of either causes an increased elimination of the other at the expense of the body's store, if necessary. Proportionate increase in the intake of both increases the fecal excretion. Marked deviation in the balance of calcium and phosphorus partially diverts the elimination of the more abundant through

the kidney. The excretion of magnesium and calcium are likewise interrelated.

Sweat

Next to the kidneys, the skin is, in man, the most important channel for the elimination of water. The volume eliminated varies widely under different physiological and pathological conditions. Obviously the elimination in warm weather is much greater than in cold weather, also during muscular activity than during rest. The specific gravity varies between 1.001 and 1.015, ordinarily amounting to about one-half the latter figure. The solids range from about 0.4 to 2.0 per cent. The reaction may be acid, neutral or alkaline to litmus, although under normal conditions it is most often acid. Protein is generally present in traces.

The skin excretes, qualitatively, practically the same substances as occur in the urine, namely, urea, ammonia, uric acid, amino-acids, creatinin, chlorids, phosphates and sulphates. Probably for this reason it has been more or less generally accepted that the skin and kidneys can act, to a certain extent, vicariously. At one time the use of sweat-baths in the treatment of nephritis was common. The quantity of substances excreted by the skin, however, is quite insignificant in comparison to that excreted by the kidney. In addition to their power to excrete water, the sweat glands do appear to possess the power of excreting salt, the quantity of sodium chlorid amounting to from 0.2 to 0.5 per cent.

A variety of methods have been employed to collect sweat. Probably the most satisfactory procedure is to place the patient in a rubber bag during the sweating period. Sweat obtained in this way is a cloudy, nearly colorless liquid, which settles or filters nearly or perfectly clear. In the comparatively recent experiments of Riggs, and Plaggemeyer and Marshall this was the method employed. In his work on the cutaneous excretion of nitrogen, where an attempt was made to determine the twenty-four hour excretion, Benedict extracted the nitrogen from specially prepared underwear.

An idea of the composition of sweat obtained from normal subjects and nephritic patients may be obtained from the table on the next page compiled from the observations of Riggs. The sweat was obtained by placing the subject without clothing in a rubber bag which enclosed the entire body except the head. Sweating was induced by covering with a pack of hot blankets for thirty to forty-five minutes.

The observations on the nephritic patients are not especially significant. It is of interest, however, that in the first two cases where the volume of sweat is large the percentage of nitrogen is low and the chlorids high, whereas in the last two cases where the volume is small, the reverse is true.

COMPOSITION OF HUMAN SWEAT

Specimen and Subject	Quantity	Nitrogen			Urea Plus Ammonia Nitrogen Terms of Total N	Total Solids	Sodium Chlorid
		Total	Ammonia	Urea			
	c.c.	%	%	%	%	%	%
1. Normal	216	0.074	0.006	0.035	57	0.49	0.36
2. Normal	117	0.077	0.007	0.049	73	0.51	0.34
3. Normal	246	0.050	0.007	0.026	66	0.39	0.25
4. Normal	96	0.126	0.007	0.069	60	0.59	0.36
5. Normal	170	0.085	0.006	0.049	58	0.56	0.33
6. Normal	140	0.083	0.006	0.040	55	0.55	0.35
7–16. Nephritic on regular diet	324	0.064	0.054		82	0.52	0.46
17–23. Nephritic	221	0.077	0.054		69	0.65	0.53
24–26. Nephritic	90	0.215	.....		..	0.24	0.12
27–29. Nephritic	77	0.158	0.116		65	0.43	0.15

The total nitrogen content of sweat appears to vary from 0.05 to 0.20 per cent, from 50 to 80 per cent being in the form of urea and ammonia. According to the observations of Benedict(*a*) the average daily loss of nitrogen in the perspiration when the subject performs no muscular work amounts to 0.07 gram, but during hard muscular work as much as 0.2 gram may be excreted in a single hour.

From the data of both Riggs and Plaggemeyer and Marshall the urea content of sweat appears to amount in round numbers to 0.1 per cent. As the latter workers have pointed out, the relationship between the different forms of nitrogen in sweat and urine are entirely different. The concentration of urea in sweat is from three to ten times as high as that of the blood but only one-tenth the concentration in the urine.

Uric acid occurs in sweat in much smaller amounts than in either blood or urine, the concentration being about one-twentieth that in blood and one-five-hundredth that in urine. If creatinin is present it exists in very small amounts.

The greater part of the total solids is made up of sodium chlorid, although according to the observations of Riggs sufficient potassium is present to combine with twenty per cent of the chlorin. For example, with a solid content of 0.5 per cent one might expect a salt content of 0.35 per cent. The salt excreted in the sweat may readily amount under certain conditions to two or three grams per day, a quantity ten times that normally present in the feces. Phosphates are present only in traces.

A diastatic ferment is present in the sweat in appreciable amount. Such dyes as phenolsulphonephthalein are not excreted by the skin nor does the injection of phlorhizin result in the excretion of sugar by the sweat glands.

SECTION V

Normal Processes of Energy Metabolism. . . . . *John R. Murlin*

Indirect Calorimetry—Methods of Measuring the Respiratory Exchange by Means of a Respiration Chamber—Methods for Measuring the Respiratory Exchange by Direct Connection with the Respiratory Passages—Methods of Calculating the Heat Production from the Respiratory Exchange—The Non-protein Respiratory Quotient—Direct Calorimetry—The Heat of Combustion—Animal Calorimetry—Basic Principles of Energy Metabolism—The Energy of Muscular Work Is Definitely Related to the Potential Energy of the Food—The Energy Metabolism Is Determined in Part by the Environing Temperature—The Indigestion of Food Increases the Metabolism—Basal Metabolism—Energy Metabolism of Growth—Energy Metabolism of Pregnancy—Energy Metabolism of the Newborn Infant—Energy Metabolism from Two Weeks to One Year of Age—Energy Metabolism of Children up to Puberty——Energy Metabolism of Old Age.

Normal Processes of Energy Metabolism

JOHN R. MURLIN

ROCHESTER

It is a familiar fact that the temperature of what we call "warm-blooded" animals is not only several degrees higher than the average temperature of the atmosphere, but it is held constantly at this level despite fluctuations of the environing temperature. So-called "cold-blooded" animals likewise produce heat, the difference being that in these the body temperature is not regulated but is dependent upon the external temperature. All animals therefore are transformers of energy. In fact experience and theory are in accord in regarding the production of heat as a necessary consequence of the phenomena of life; it is a sign, indeed, of vital activity.

There are two general methods of measuring the production of heat: (1) by determining the intensity of the chemical processes (combustion) by which heat is liberated in the organism; and (2) by registering directly the heat disengaged by the organism in a calorimeter. The first is known as the indirect or chemical method; the second, the direct or physical method.

A. Indirect Calorimetry

The indirect or chemical method depends upon the successful measurement of the respiratory exchange. We must, therefore, consider at some length the technology of this subject. In the meantime it may be stated that the indirect method of calorimetry offers certain advantages over the direct method. When the latter subject is considered (page 567) it will be evident that in order to measure all of the heat discharged from the animal body by the several routes of escape a rather complicated apparatus is necessary. In time this may be simplified, but at present an accurate calorimeter is far more complex and far more costly both in initial cost and for operation than a respiration machine. Secondly, the indirect method is more accurate as matters now stand. Krogh(*c*) finds that he can measure oxygen absorption with his micro-respiration apparatus to an accuracy of 2 cu.mm. of O_2, equivalent to 10

milligram-calories in ten hours, while the highest accuracy attainable by Bohr and Hasselbalch with their egg calorimeter was 100 milligram-calories. The percentage difference is not so great as this in applying the two methods simultaneously to the study of the human organism; but one comes very soon to rely upon the indirect measurement more than the direct (see page 586). Furthermore, and in the third place the two methods agree very closely in the best forms of respiration calorimeters. This being true and the indirect method being both simpler and more reliable, greater space will be given to its description and to the methods of calculating energy production from the fundamental data, than for the direct method.

I. Methods of Measuring the Respiratory Exchange by Means of a Respiration Chamber

The methods of measuring respiratory metabolism are of two general kinds: (a) one requiring a chamber in which the subject is confined, and (b) a method so devised that the respiratory passages are connected directly with the measuring apparatus.

Two general types of ventilation also have been used, one known as the open-circuit and the other as the closed-circuit type. The classical instance of the first type is the apparatus of Pettenkofer first described in 1863 and later improved by C. Voit. The classical instance of the closed-circuit type is the Regnault-Reiset apparatus first described in 1849. Only the more important constructions of each type will be described here.

1. Open-circuit Type of Apparatus.—a. *Pettenkofer Apparatus.*—The original apparatus of Pettenkofer consisted of a chamber containing 12.7 cubic meters which was ventilated by means of air pumps drawing air from the outside. The air was aspirated through the chamber and at the point of exit samples were measured after having been passed through pumice stone saturated with sulphuric acid thence through barium hydrate for the absorption of the carbon dioxid. In the earliest experiments performed with the apparatus by Pettenkofer the efficiency of the absorption system was checked by burning candles in the apparatus and an error of 1.96 per cent was found as the average for a considerable number of tests. The error on the water absorption was somewhat higher, varying from 2.5 to 3.5 per cent.

This apparatus was used exclusively with the human subject. For obtaining the oxygen absorption Pettenkofer and Voit(*c*) employed the following method: Adding to the original weight of the subject the amount of food consumed and the amount of water drunk a sum was obtained which was subtracted from the final weight of the subject plus all of the excreta (urine, feces, carbon dioxid and water vapor). The difference between these two sums was taken as the oxygen absorption.

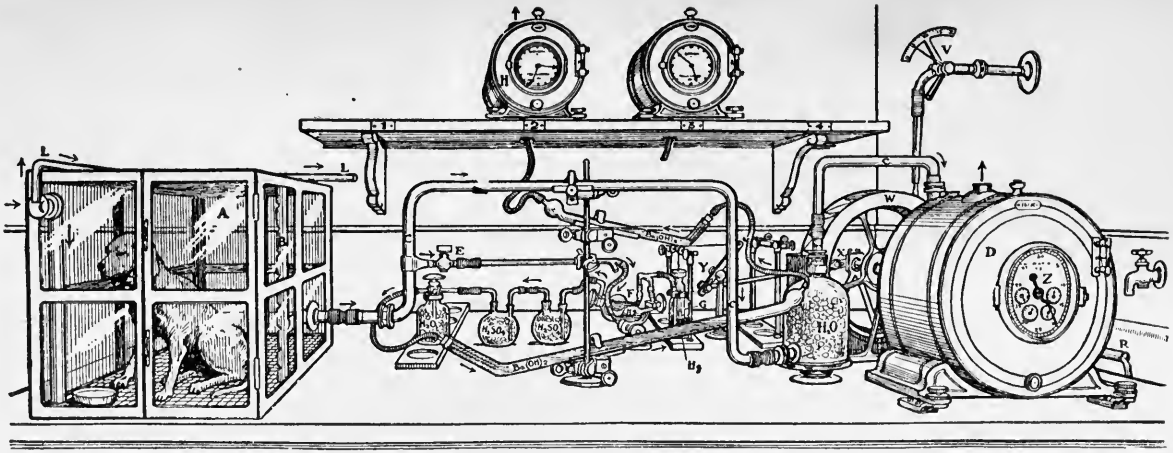


Fig 1. The smaller respiration apparatus of Pettenkofer and Voit. Air enters the cage, *A*, at the upper left-hand corner. It is drawn out through openings in the tube *B* (so spaced as to insure thorough diffusion) through the tube, *C*, and the large H_2O flask, where it is saturated with moisture, to the gas meter, *D*, where it is measured. The meter is driven by a water wheel, *W*, rotated at a uniform rate of speed by a constant head of water pressure, *V*. At *E* a side tube leads off from *C* conveying a sample of cage air for analysis. This air is drawn through a mercury valve, *F*, by means of the mercury pump, *G*. The latter is operated by the mechanism, *X*, *Y*, connected with the water wheel. From the valve, *F*, this air passes through two H_2SO_2 flasks and is then saturated with moisture so as to prevent loss of water from the $Ba(OH)_2$ tubes. Of course the air as it leaves the $Ba(OH)_2$ tubes is saturated with moisture, and is measured by the small gas meter, *H*, just as in the case of the large meter, while in this saturated condition. A duplicate sample is led off at the same time through another branch of the tube, *E*, and through another system of the vessels to the gas meter at 3. Through the branches of the tube, *L*, duplicate samples of the air which enters the cage are drawn in the same manner to similar valves, and then through similar vessels and tubes to meters placed at 1 and 4. (From Tigerstedt, New York, D. Appleton & Co.)

In the modified form of apparatus devised by Voit(*d*) for experiments on small animals the suction pumps were replaced by a large meter driven by a water wheel which served at once to aspirate the air through the chamber and to measure its volume. The chamber devised by Voit was of small capacity containing only 64 liters. Larger chambers, however, were used as, for example, the chamber in the accompanying figure which had a capacity of 340 liters.

The construction of the small suction pumps also was somewhat modified in the Voit construction and a very useful type of valve with mercury seal known as the Voit valve was employed to give direction to the air sample. (See figure 1.) With this type of apparatus in five control experiments in which pure olein was burned in the form of a candle or tallow dip, an average error of 1.75 per cent was found for the CO_2, and for the absorption of water an error which varied from 1.4 to 5.5 per cent.

Wolpert(*a*) working under the direction of Rubner also made some improvements on the Pettenkofer type of apparatus. His chamber measured 1.5 x 2.5 x 2 meters with a cubic capacity of 7.5 cubic meters. The measuring drum was driven by means of a water motor. The apparatus differed otherwise in only minor details from the Voit construction, but Rubner(*j*) succeeded in measuring the water vapor with a much greater degree of accuracy.

b. *The Apparatus of Sondén and Tigerstedt.*—This apparatus erected at Stockholm and first described in 1895 was so constructed as to accommodate a number of individuals employed as subjects at the same time. The chamber consisted of a room measuring 5 x 5 x 4 meters and had a total capacity of approximately 100 cubic meters. The walls were sealed with sheet metal carefully soldered together and the room was ventilated through a zinc pipe measuring 14 cm. in diameter which was carried up above the roof of the room and capped with a ventilator containing a valve to guard against aspiration of air from the room by action of the wind. The room was heated by steam and the air was kept stirred by means of an electric fan. Ventilation was accomplished by means of pumps gauged to three different speeds which could be adapted to the number of individuals serving as subjects. Samples of air were withdrawn from the exit tube near its mouth and were analyzed by means of the Sondén-Petterson apparatus. Check experiments with burning candles or petroleum gave an average error of 1.16 per cent on the CO_2. In other series of experiments performed later by Rosenberg the error was reduced to 1 per cent. This apparatus and a later one on the same principle at Helsingfors (Tigerstedt(*g*)) have been used especially for the study of metabolism in school children.

c. *The Apparatus of Atwater and Rosa.*—This apparatus constructed with the aid of the U. S. government in the chemical laboratory at Wesleyan University, Middletown, Conn., was first described in 1897. It con-

sisted of a chamber 2.15 x 1.22 x 1.92 meters or a cubic capacity of 5.03 cubic meters. It was ventilated by means of a so-called Blakeslee pump of a reciprocating type. By means of a toothed wheel containing 100 teeth, the first and fiftieth of which were longer than the others, samples of air could be diverted from the main stream at each fiftieth stroke of the pump. These samples were collected in pans for analysis.

The apparatus was not long used in this form. Realization of the necessity for accurate determination of the oxygen absorption led to its modification to the closed-circuit type as will be described later.

The apparatus was at once a respiration chamber and a calorimeter for direct measurement of the heat. The method of heat measurement will be described in a later section.

d. *Apparatus of Jaquet.*—This apparatus in its original form has a cubic capacity of 1393 liters. The subject can either sit or lie down during the observation. It is ventilated by means of a bellows driven by a water motor, the air being withdrawn from one end through an exit tube and being replaced by pure air from the outside which enters at the other end. The air is passed through a gas meter after withdrawal from the apparatus. Samples are aspirated from the exit tube by means of a mercury pipette, the leveling bulb being lowered by means of a pulley connected with the axle of the measuring meter so that the rate of sampling is proportional to the rate of ventilation.

The air analyses for CO_2 and O_2 are accomplished by means of the Petterson apparatus.

Precaution against change of composition of air in the apparatus is taken by analysis of the air just before the beginning and just at the end of an observation period.

By burning alcohol in the apparatus an average error of 1.8 per cent was attained. An experimental period could be prolonged with this apparatus for some 12 to 13 hours.

e. *Apparatus of E. Grafe(b).*—This is a modification of the Jaquet type of apparatus so constructed as to accommodate a man in a standing, sitting or lying position. The respiration chamber consists of a rectangular base bordered by a groove into which the superstructure of the chamber is made to fit air-tight by means of a liquid seal. The whole upper part of the chamber is suspended from the ceiling of the room by means of pulleys and a counterpoised weight. Entrance to the apparatus is gained by raising one end of the superstructure. The rectangular section of the apparatus measures 0.9 meter at the head and foot ends and 2 meters in length. In the vertical section one end is higher than the other, measuring 1.7 meters at the head end and 0.75 meter at the foot end. The frame is constructed of wood covered with sheet metal painted with an oil paint.

Ventilation of this apparatus is accomplished in exactly the same manner as in the original Jaquet construction, air being drawn through and

measured simultaneously by means of a gas meter driven by water power. Samples taken by the aliquot method of Jaquet are analyzed for oxygen and CO_2 by means of the Petterson analyser.

The apparatus used by Krogh and Lindhard at Copenhagen is of the Jaquet-Grafe type (Fig. 2).

f. *Apparatus of Haldane(a).*—A convenient form of open circuit type of apparatus devised for observations on small animals is that of Haldane described in 1892. The respiration chamber (Figure 3) consists of a large

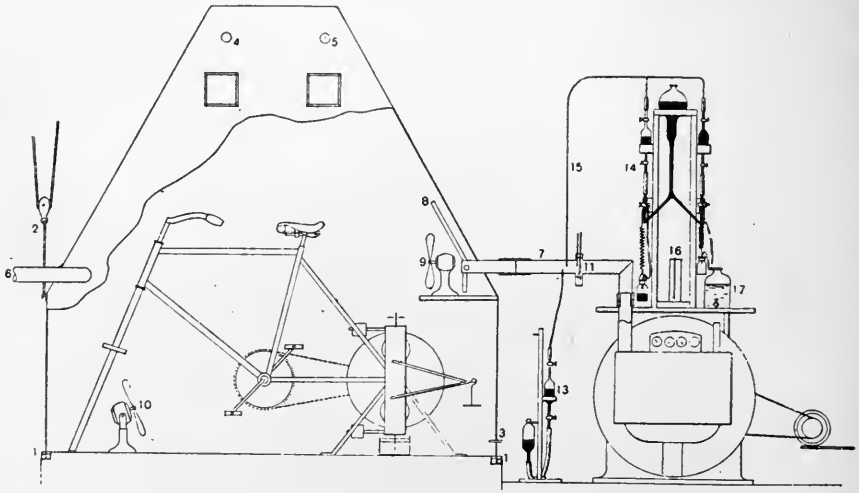


Fig. 2. Diagram of the Jaquet-Grafe respiration apparatus used by Krogh and Lindhard. The floor is made from a single sheet of galvanized iron with the edges bent down into a U-shaped rectangular groove (1) which is filled with water. As shown at (2) one end can be lifted to let in the subject and put in the apparatus; (3) small tubes introducing wires, etc., for the working of the ergometer; (4 and 5) ventilating tubes for use with a meter; (6) inlet for outside air; (7) side tubes drawing air from points 50 cm. from the outlet; (9 and 10) fans for mixing the air; (11) wet and dry bulb thermometers; (12) bottle of water keeping water level in the meter; (13) hand sampling apparatus; (14) automatic sampling apparatus; (15) tube leading from outlet to the automatic sampling apparatus; (16) thermometer in the meter.

bottle of 16 liters capacity. Air is aspirated through the bottle by means of an ordinary laboratory water suction pump. The ingoing air is passed over sulphuric acid in pumice stone and another bottle containing soda lime. The outgoing air is likewise passed through three absorbers, the first containing sulphuric acid, the second soda lime and the third sulphuric acid. The gain in weight of the first gives the amount of water vapor exhaled by the animal. The gain in weight of the second two gives the amount of carbon dioxid exhaled. After passing the absorbers the air is again saturated with moisture and measured by a gas meter.

The apparatus is of such a size that the chamber with the contained animal can be weighed. Loss in weight of the animal during an experi-

ment less the gain in weight of the absorbers gives the amount of oxygen absorbed.

2. Closed Circuit Type of Apparatus.—In most of the open-circuit types of apparatus thus far described the determination of oxygen is indirect, being based upon the loss of body weight of the subject. The absorption of oxygen can be determined directly, however, provided the subject is enclosed in an air-tight system of known capacity. The simplest system of this sort consists of a respiration chamber only of large enough capacity to supply oxygen and permit respiration of ordinary atmospheric air without discomfort for at least an hour. By analysis of a sample of air at the beginning and the end of an observation it is possible to learn from the changed composition the amount of oxygen absorbed and the amount of CO_2 given off.

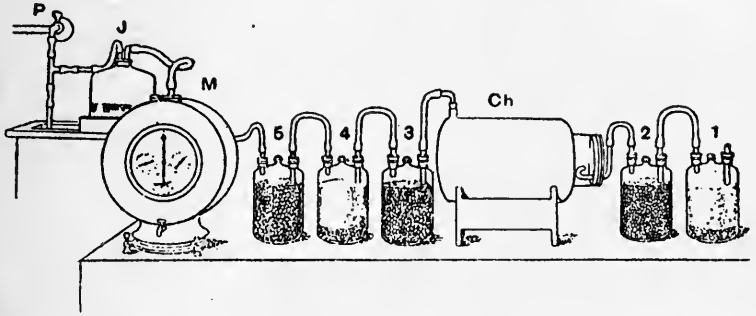


Fig. 3. Haldane respiration apparatus. Ch, chamber; 1 and 2 absorbers for ingoing air; 3, 4, and 5, absorbers for outgoing air; M, meter; J, safety bottle; P, air pump.

A more physiological arrangement, however, is to provide for the absorption of the carbon dioxid approximately as rapidly as it is produced and its replacement by oxygen. The observations can then be prolonged for many hours.

a. *The Apparatus of Regnault and Reiset.*—This is the original closed-circuit apparatus. The respiration chamber consists of a glass bell of 45 liters capacity (A, Fig. 4). The bell is fitted by an air-tight seal into a metal base which serves at the same time as the base for the surrounding water jacket. Entrance to the chamber is gained by means of a circular opening in the base. The top or handle of the bell is perforated by several tubes one of which connects with a mercury manometer (a, b, c) for recording the pressure inside the chamber. A second connects with a sampling apparatus d′. Two others connect with the CO_2 absorbers C and C′, and a fifth with the oxygen supply (the flasks N, N′ and N″). The CO_2 absorbers have a capacity each of about three liters. The absorbing fluid is an assayed solution of KOH. Movement of air from the chamber to the absorbers is accomplished by alternately raising and lowering the absorbers. For example, when C is raised as in the figure the fluid runs

from C into C′, thereby aspirating the air into C and returning air from C′ to the respiration chamber. By thus absorbing the CO_2 produced by the subject the volume of the contained air is reduced and its place is taken by oxygen driven from the flask N by water pressure. The experiment is continued until all the oxygen contained in the three flasks is used up. The last 300 or 400 c.c. of oxygen is driven over under pressure and the experiment is continued until the atmospheric pressure is again reached. At this moment samples of the chamber air are drawn off for analysis.

The CO_2 is discharged from the KOH by weak sulphuric acid and is again caught in a KOH absorber to be weighed. It could not be obtained

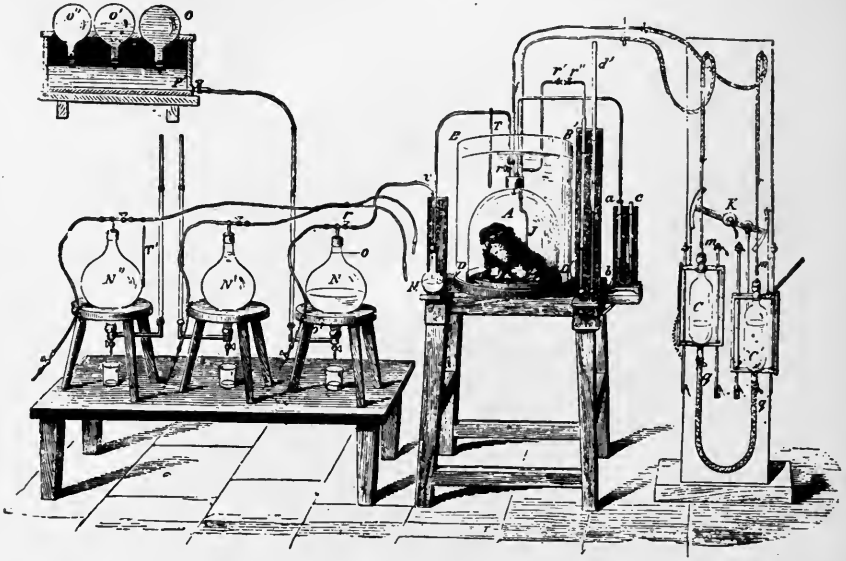


Fig. 4. Respiration apparatus of Regnault and Reiset. *A*, chamber for animal; *B*, water jacket; *C*, carbon dioxid absorbers; *a*, *b*, *c*, manometer for recording pressure inside respiration chamber; *N*, *N′*, *N″*, flasks containing oxygen; *T*, *T′*, thermometers.

by direct weighing of the absorbers because they contain some water exhaled from the animal as well as CO_2. To the amount of CO_2 contained in the KOH is added the residual amount found in the chamber air by analysis at the end of the observation.

The oxygen absorbed is found by measurement of the contents of the flasks corrected by analysis of the chamber air.

b. *The Apparatus of Hoppe-Seyler*(*c*).—Similar in principle to that of the original construction of Regnault and Reiset this apparatus consists of a horizontal cylinder two meters in length, 1.66 meters in diameter and a total capacity of 4.480 cubic meters. It is, therefore, large enough for observation on the human subject.

The respiration chamber rests on the ground floor of the laboratory,

the driving mechanism, absorbers and gasometers being set up in the cellar immediately below the respiration chamber (Fig. 5). The air of the chamber is cooled by means of a stream of water passing through a grid of pipe placed near the ceiling of the chamber. Besides the main ventilating tubes which connect with the CO_2 absorbers (b) other tubes penetrate the walls of the apparatus for recording the internal pressure, for admitting oxygen and for withdrawing a sample of air for analysis. The CO_2 absorbers are alternately raised and lowered by means of a walking

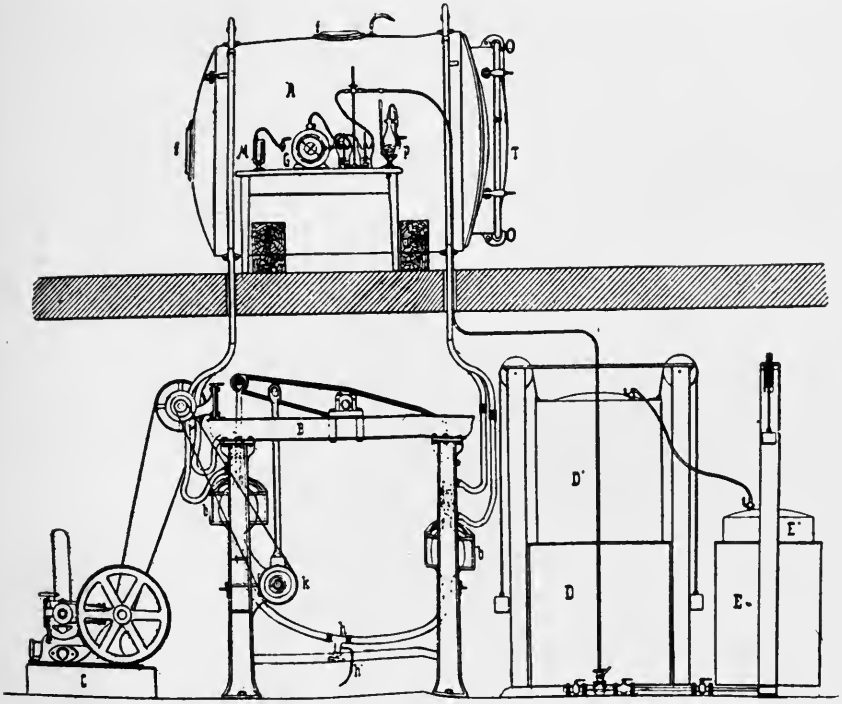


Fig. 5. Respiration apparatus of Hoppe-Seyler. *A*, respiration chamber; *B*, apparatus for raising and lowering carbon dioxid absorbers; *C*, engine; *D*, gasometer filled with oxygen; *G*, meter for measuring sample.

beam operated by a gas motor. Air is thereby alternately withdrawn and returned to the chamber after absorption of its carbon dioxid.

Oxygen is admitted from the gasometer D through a gas meter G after passing first through a water flask to prevent evaporation of water from the meter.

The carbon dioxid absorbed is determined exactly as in the Regnault-Reiset method by discharging the CO_2 from the KOH and collecting it again and weighing. This amount obviously must be corrected by analysis of the air residual in the chamber at the end of an observation.

Oxygen is determined by reading the gas meter and correcting the

amount so indicated by the residual analysis. The quality of the oxygen supplied is likewise controlled by analysis.

c. *Apparatus of Atwater and Benedict(d).*—These authors introduced the use of an eccentric blower (Fig. 6) for driving the air through the absorption system and back to the respiration chamber. The original chamber described on page 518 for the open-circuit apparatus was adapted to the new type of ventilation shown in Fig. 6. In the upper part of the figure the respiration chamber is shown and below it is the blower and absorbing or purifying system. Air from the chamber containing nitrogen, carbon dioxid, water vapor and a somewhat diminished percentage of oxygen passes through the blower and enters the absorption system. Here it is forced through sulphuric acid to remove the water vapor and through a specially prepared soda lime which takes out the carbon dioxid; the soda lime, however, contains water some of which is taken up by the dry air. A second sulphuric acid absorber to catch this water is therefore necessary and the total CO_2 absorption is found by the gain in weight of these two vessels. The air is now freed of carbon dioxid and water, but is still deficient in oxygen. The latter in requisite amount is admitted from a cylinder of compressed oxygen through an opening in the ventilating pipe (see Fig. 6) and the air now restored to its original composition re-enters the respiration chamber.

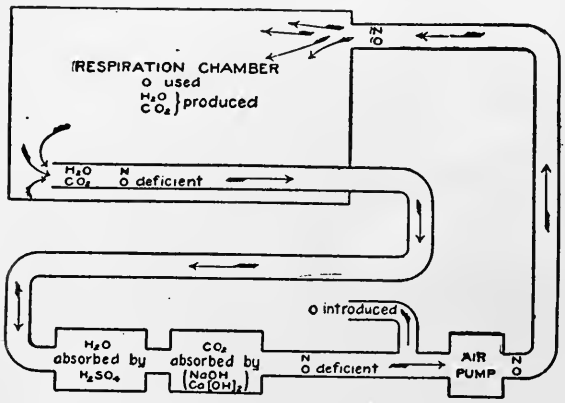


Fig. 6. Diagram of the system of ventilation in the closed-circuit apparatus of Atwater and Benedict. The direction of the air is indicated by arrows.

The respiration chamber of the original construction continued to be used as a calorimeter. In later patterns of this respiration calorimeter which have been constructed at the Nutrition Laboratory of the Carnegie Institution at Boston (Benedict and Carpenter(*a*)), at Cornell Medical College (Williams, H. B.) and at the U. S. Department of Agriculture (Langworthy and Milner) some slight modifications of the original plan have been made and these will be described here so far as the arrangements for ventilation and determination of the respiratory exchange are concerned as if belonging to the original construction at Middletown.

The metal walls of the chamber and the ventilating pipes which consist of metal or heavy rubber confine the air to a definite volume and to allow for expansion or contraction of the air volume as the result of pres-

sure and temperature changes a compensating device in the form of a spirometer is inserted (see Figs. 7 and 8).

The approximate amount of water vapor coming from the subject's body and the amount of carbon dioxid exhaled from his lungs is found by direct weighing of the absorbers. Likewise weighing of the oxygen cylinder gives within a small margin the amount of oxygen absorbed by the subject. These amounts would be absolutely correct if there were no change in barometric pressure or temperature of the confined air, and if the composition of the air at the end of an observation period were exactly the same as at the beginning.

Barometric pressure and temperature are readily determined from accurate instruments and corresponding corrections in the volume of the contained air are readily made. For detecting alterations in the composition of the air resulting from inefficiency of an absorber or from unusual production of CO_2 or water vapor, known volumes of the circulating current are diverted from the main pipe and are made to pass through a smaller channel over sulphuric acid and soda lime and sulphuric acid again (exactly as in the main circuit) contained in U tubes which can be weighed to a high accuracy on a sensitive balance (Fig. 8).

As an illustration of a compact form of this apparatus constructed for determination of the respiratory exchange alone (without direct measurement of the heat) either in laboratory animals or in infants, the design of Benedict and Talbot may be described.

This apparatus was originally described by the authors in a preliminary publication in 1912. Later it was somewhat modified and was employed in most of their observations on the infant in the form shown in Fig. 7. In this form it was capable of determining the oxygen directly, exactly on the same principle as that described above for the respiration calorimeter.

The chamber C, in which the infant reposes, is provided with a water jacket, W. W. for temperature control. The air leaves the chamber (Fig. 7) near the right hand end at O, and is drawn by the rotary blower over a wet and dry bulb psychrometer, Z, which gives the amount of moisture in the air of the chamber. A can, N, filled with dry cotton batting is also placed in the air-current between the blower and the chamber to act as a muffler. After leaving the exhaust side of the blower, P, the air is forced through an empty glass bottle, A, which serves as a trap should any back-pressure take place and sulphuric acid be forced back from the water-absorbing vessels, B and C. These latter vessels are of peculiar construction. They were designed by Williams for the small respiration calorimeter at Cornell Medical College. The air passes along a pipe to a 2-way valve, V, where it may be deflected through either of the soda lime bottles D_1 or D_2 in which the carbon dioxid is absorbed. Since the reagent must be somewhat moist to facilitate the absorption it gives up water-vapor to the dry air-current, which must in time be absorbed by sulphuric acid in the Wil-

liams bottles E_1 or E_2. The air next passes through the 2-way valve, V_2, and enters a small can, F, which contains dry sodium bicarbonate, where the unweighable and nearly imperceptible sulphuric acid odors are effectually removed. The air then returns to the chamber through the by-pass J, or, if it is desired to moisten the air, the current can be deflected by closing the valve, R, in the bypass, J, so as to pass all of the air through distilled water in the Williams bottle K. The air is now free from carbon dioxid and contains the water vapor added in passing through K, but is still deficient in oxygen. This deficiency is made up by admitting oxygen from the pressure cylinder L. The air thus enters the respiration chamber I somewhat moist and with approximately normal percentage of oxygen.

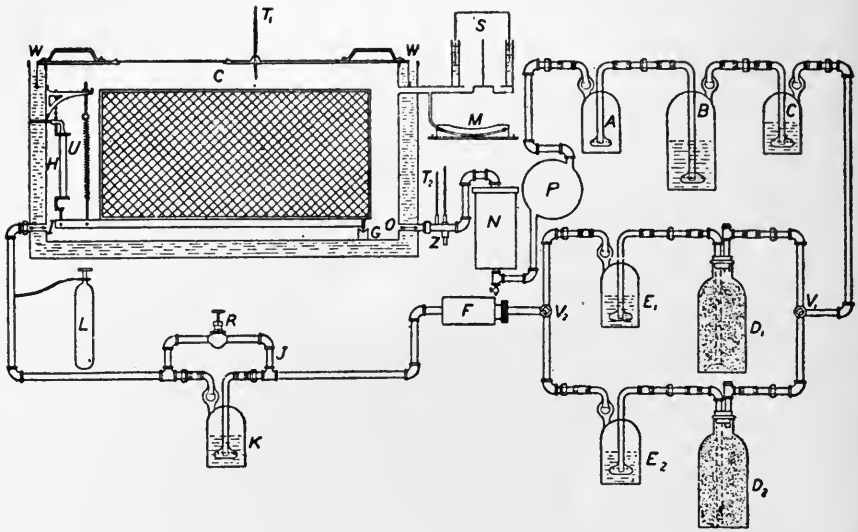


Fig. 7. Diagram of the respiration apparatus used by Benedict and Talbot in their study of the gaseous metabolism of infants. Description in the text.

Either series of absorbers may be used as desired, for if the air current has been passing through the series D_1 and E_1, for a given experimental period, the air can be instantly deflected through the series D_2 and E_2 by turning the valves V_1 and V_2. These valves are connected by a long rod so that they may be thrown simultaneously by one movement of the hand.

Since the air-current is entirely closed a small spirometer S is attached at the upper right hand corner of the respiration chamber, thus providing for an expansion or contraction of the air. A thermometer, T_1, in the cover of the chamber and a second thermometer, T_2, in the outgoing air serve to indicate the temperature changes while the manometer, M, shown below the spirometer indicates the pressure of the air in the chamber.

By noting the increase in weight of the absorbers D_1 and E_1 or D_2 and E_2 the amount of CO_2 absorbed is known. It is possible that the amount

of water vapor given up by D_1 or D_2 to the dry air passing through it may be actually more than the amount of carbon dioxid absorbed, or that the bottle D_1 or D_2 may be losing weight; on the contrary, the water vapor given up is immediately absorbed by E,, and hence the algebraic sum of the difference in weight of the two bottles gives the weight of the carbon dioxid absorbed. Usually both bottles are weighed on a balance at the same time. The loss in weight of the cylinder, L, gives the amount of oxygen absorbed by the subject, corrections being made for any variation in temperature and barometric pressure. Corrections for changes in composition of air inside the chamber may be made by withdrawing samples through a by-pass not shown in the figure.

The infant is placed inside a wire crib supported at one end upon a stout spiral spring, U, and at the other end upon a knife edge, G; this mode of suspension affords a means of recording the muscular activity of the infant. Alongside the spring, U, is a pneumograph, H, the distention or contraction of which compresses the air inside of the pneumograph tube, thus transmitting to a delicate tambour outside a record of the lightest motion of the cage.

The respiration chamber is constructed of galvanized iron or copper, and is 77 cm. long, 25 cm. deep, and 37 cm. wide. To insure temperature control the whole respiration chamber is surrounded by a water jacket consisting of a second shell of galvanized iron or copper with a space of 5 cm. between the two walls. The water jacket which is filled with water to within a few centimeters of the top acts also as a seal when the cover is placed upon the apparatus. In the cover are a window securely fastened and an opening for the air thermometer.

The psychrometer is essential for indicating the degree of moisture inside the respiration chamber. This is of value not only for the comfort of the infant, but also for computing the amount of oxygen inside the chamber at the end of the experimental period. Experiments carried out with a very delicate instrument have shown that the depression of the wet-bulb thermometer can be measured with great accuracy and the amount of water vapor in the air computed with an exactness sufficient for all practical purposes. The two thermometers are graduated to 0.1° C. but are capable of being read with a lens to .02° C. It is necessary to make sure that the cloth around the wet bulb thermometer is kept thoroughly drenched with distilled water, also that the capillarity of the fiber is good as otherwise the cloth may become partially dried and inaccurate results obtained. Prior to each experiment the wet bulb is drenched by using an elongated medicine dropper filled with distilled water.

The blower, P, is connected with a leather belt to a small electric motor and can be provided with a safety clutch to prevent reversing the wheel through carelessness, and the drawing over of sulphuric acid from the water absorbers. The safety trap, A, is an additional security against this mis-

hap. The blower used with this apparatus gives a ventilation of about 35 liters of air per minute when rotating at a speed of 270 revolutions p. m. Experiment with an alcohol flame shows that this rate of ventilation does not produce a draft which would be perceptible by the infant. The fact that the relative humidity does not become unduly low, even without use of the water bottle, is proof that the infant is sojourning in an atmosphere approximately normal.

To remove the moisture coming from the lung and skin of the infant, and any additional moisture from water bottle K, one large-sized Williams bottle B is usually sufficient. However, a second bottle C removes the last traces of water vapor. To facilitate the handling of these bottles in weighing and to prevent breakage, they are usually enclosed in a small wire basket with a handle by means of which they may be suspended directly from a hook on the arm of the balance.

The Williams bottles as well as the soda lime bottles are fitted with short lengths of rubber tubing of good quality to which are attached respectively male and female parts of ordinary garden hose couplings of standard ¾ inch size; with a standard rubber hose gasket, the couplings are made air-tight by a single twist of the hand.

For infants weighing not less than 3 to 5 kgm. the soda lime container holding in the neighborhood of 2 kgm. soda lime is capable of absorbing all the carbon dioxid. This amount of soda lime will take up as much as 75 gm. CO_2 without renewal.

The direct determination of oxygen may be made either by weighing the small cylinders of gas L, and noting its loss in weight during the experiment, or by passing the gas, under reduced pressure, through a delicate and accurate gas meter. With oxygen made from liquid air a corrective for argon has usually to be made amounting to about 1 per cent. The volume of air inside the respiration chamber is about 75 liters. Correction for temperature change is therefore necessary in order to determine the actual volume of air at the end of every experimental period. Two carefully calibrated mercury thermometers, one in the cover of the chamber, the other the dry bulb thermometer of the psychrometer, are used to record such changes. While the two thermometers barely read alike, their fluctuations are usually parallel. The average of the readings of the two is taken as representing the average temperature of the air in the chamber.

It is important that the respiration chamber shall not be subjected to sudden fluctuations of temperature during the experimental periods. The water-jacket serves to damp any changes in the room temperature, and by supplying either heat or cold to maintain the chamber at a temperature either above or below that of the room. During cold weather a mercury thermo-regulator placed in the water and connected with a small burner placed underneath, secures a constant temperature which may be regulated

at any desired level. In the excessively warm days of summer, it is necessary to place ice in the tank.

An apparatus devised by the writer, and constructed simultaneously with the last for use in Bellevue Hospital, New York, follows the same general principles as that just described, but employs as a means of controlling the temperature the electrically regulated incubator of Freas.

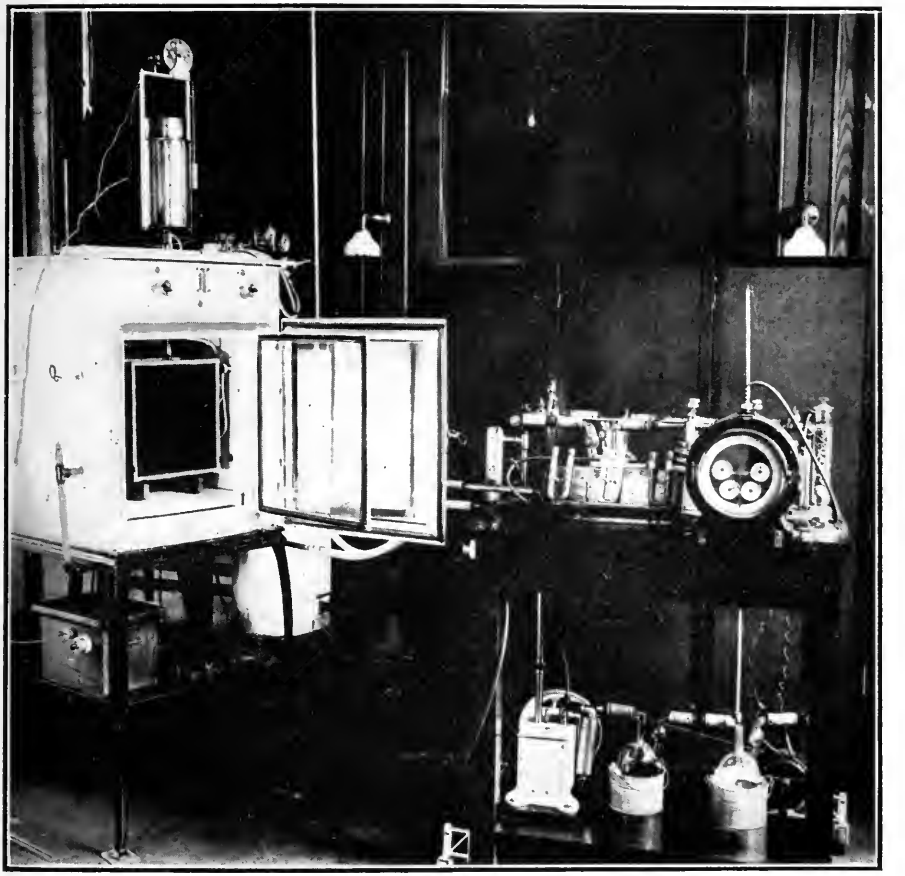


Fig. 8. Respiration incubator (Murlin).

For this reason it has been called a "respiration incubator," and can be used as an incubator for premature infants independently of its features as a respiration machine (Murlin(*d*)).

d. *Apparatus for Very Small Animals.*—With very small animals, their eggs or larval stages it is not necessary to circulate the air through absorbers. The absorption of oxygen can be recorded by a change of pressure and the carbon dioxid can be readily absorbed by means of a suitable solution of alkali. Several forms of apparatus constructed on these principles have

been invented. Some of them should be described briefly under the heading of closed-circuit apparatus.

An original form described by Thunberg was a gas-analysis apparatus of the Petterson type for the determination of very small percentages of CO_2 in which the animals to be experimented on could be introduced into the gas-measuring pipette. Any change in volume with the animal in the confined space would be due to the difference between O_2 and CO_2 given

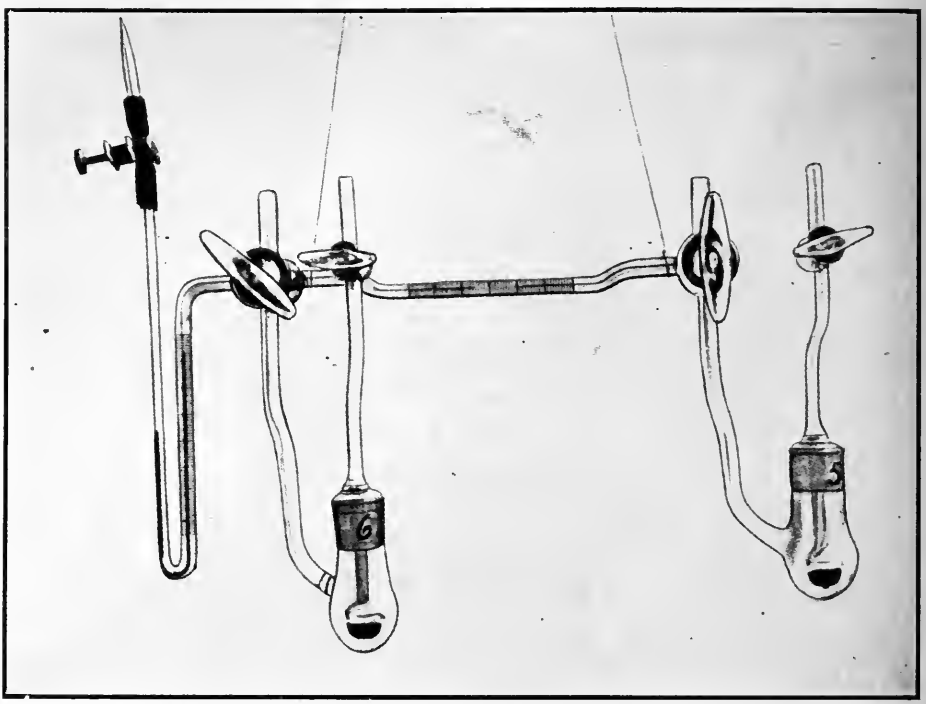


Fig. 9. Micro-respiration apparatus of Winterstein. 5 and 6, duplicate air chambers. The small animal is placed in chamber 6 and chamber 5 is used as control, the two chambers being connected by a sensitive oil manometer. The absorption of oxygen from chamber 6 is measured by the pressure of mercury necessary to restore the balance on the oil manometer.

off. This volume having been noted the air could then be driven over into potash bulb and the CO_2 absorbed. Changes in volume this time would give the CO_2 produced by the animal and the oxygen could be found by adding the difference-volume first noted.

Winterstein(*a*) improved upon this apparatus by employing the principle of the compensating vessel first introduced into gas analysis by Petterson and connecting the two vessels (the animal chamber and compensating chamber) by means of a very sensitive graduated manometer containing a drop of kerosene. The oil-drop being set at zero, the level of the mercury in the U-tube manometer at the left which is graduated in cubic millimeters is

read. By absorption of oxygen from the animal chamber the oil-drop is shifted toward that chamber and whenever a reading is taken a drop is brought back to the zero mark by means of the pressure screw on the mercury column. The volume of mercury moved upward then is equal to the volume of oxygen absorbed when corrected from the original temperature and barometric pressure to 0° and 760 mm. The carbon dioxid is absorbed as rapidly as produced by a drop of caustic soda placed in the bottom of the animal chamber, the animal of course being protected from contact with the solution. The production of carbon dioxid can be determined if, in a control period, a small amount of water is used instead of the alkali. The pressure change will then indicate the difference between the volume of oxygen absorbed and the carbon dioxid given off. If the oxygen absorption is determined just before and just after this under conditions otherwise the same, the volume of carbon dioxid will be found by substracting the difference-volume from the volume of oxygen. The respiratory quotient is then available.

It is obviously necessary to keep the two chambers in the same water or oil bath in which the liquid is sufficiently stirred so that the two chambers shall be of exactly the same temperature.

The micro-respiration apparatus of Krogh follows very similar principles. With it Krogh was able to follow the oxygen absorption of a single insect egg weighing about 2 mgm. in ten-hour periods from immediately after it was laid until the hatching of the larva (Krogh(*b*)).

II. Methods for Measuring the Respiratory Exchange by Direct Connection with the Repiratory Passages

The first observations upon the respiratory exchange of man made by Lavoisier provided for the direct examination of the expired air. A copper mask was used fitting tightly over the subject's face and by some means not clearly understood the inspired air was separated from the expired air, which was passed into alkali, thereby removing the carbon dioxid. Many different modifications of the original method of Lavoisier have been devised. Those which employ means to separate the inspired air from the expired air and provide for the collection or automatic analysis of the latter should be described under the rubric of "open circuit" or air-current types of apparatus. Other methods employ some form of "closed circuit" apparatus.

1. Open Circuit Instruments. a. *Mouth-pieces, Nose-pieces, Masks.*— For connection of the apparatus to the respiratory passages of the subject a rubber mouth-piece originally constructed by Denayrouse for the use of divers has been widely employed. It consists of a wide rubber disc which fits in between the lips and the teeth of the subject. In the middle of this disc is a 2 cm. opening leading into a rubber tube of the same size. On

the two sides of the opening are thick rubber projections which may be held between the teeth. Sometimes the mouth-piece is supplemented by a band of rubber tied around the head and pressing against the lips from the outside. In the use of this device the nose must of course be closed by some form of clip or clamp (Regnard) (Fig. 10).

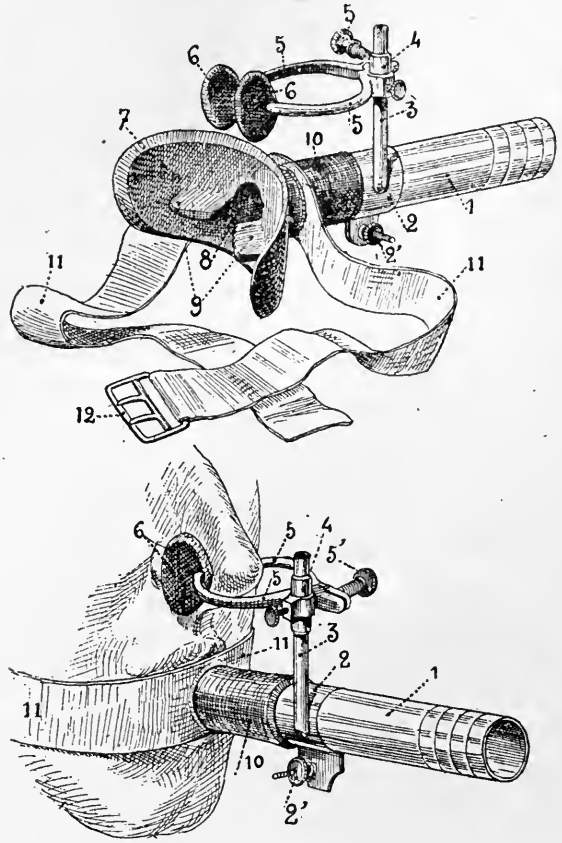


Fig. 10. Mouth-piece of Denayrouse with nose clip attached. (1) brass tube connecting to apparatus; (2) collar supporting stand (3) which in turn supports nose piece; (4) brass collar; (5) frame of nose-piece with adjusting screw for regulating pressure on nose; (6) nose pads; (7) rubber of mouth-piece which fits in between teeth and lips; (8) opening from mouth-piece into brass tube; (9) rubber lugs which may be grasped between the teeth; (10) rubber tube continuous with mouth-piece; (11) strap for holding mouth-piece firmly in place.

Glass nose-pieces have been described by Tissot and these have been improved by Carpenter(*a*). A pneumatic nose-piece described by F. G. Benedict(*d*) (Fig. 11) is much to be preferred to the all-glass construction. They can be made very secure by inflation of the pneumatic portion particularly if the outer rubber which fits against the nose is covered with mucilage. Many subjects, however, find the nose-pieces quite uncomfortable and prefer the mouth-piece described above. Benedict himself has recently recommended the mouth-piece with a clinical respiration apparatus in preference to the nose-piece (Benedict and Collins). When nose-pieces are used the mouth should be sealed shut with an adhesive tape.

Various types of masks have also been used from the crude copper mask covering the entire face employed by Lavoisier, to the modern so-called half mask employed in mine rescue work. The gas masks, perfected from force of necessity during the recent war, have also found a useful field in con-

nection with respiration experiments. A form of mask described by Bohr consists of a funnel-shaped piece of tin plate coated on the edges with a substance used by dentists, known on the market as Stent's compound. This substance softens at a temperature a little above the body temperature and may, therefore, be molded to fit the face of each subject. The mask can be made perfectly air tight by covering the molded surface with vaseline or lanolin and binding it securely to the face (Krogh(*c*)).

The half mask employed by Boothby is made of rubber on a flexible wire frame so that it may be bent to conform to the shape of the nose, cheeks and chin. It is bordered by a pneumatic cushion. Boothby finds that it is much safer not to inflate this cushion for the air-valve tends to leak, thus altering the pressure against the face and causing leakage. He recommends the use of tapes fastened to a towel which lies upon the pillow under the neck of the subject. The tapes may be drawn forward and tied about the mask transversely and obliquely in such a way as to apply the pressure just where it is most needed. (Boothby and Sandiford.) (Fig. 12.)

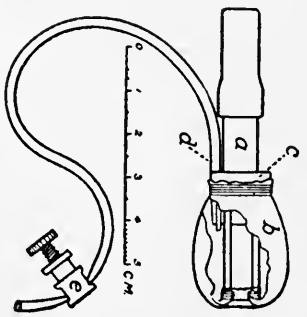


Fig. 11. Pneumatic nosepiece of Benedict. *a*, glass tube to which is fastened a rubber finger-cot, *b*, which is drawn over a rubber stopper, *c*. A capillary rubber tube, *d*, serves for dilating the cot *b*; the clamp *c* closes *d* after *b* is inflated.

Hendry, Carpenter and Emmes have shown that the oxygen consumption is practically identical with the different types of breathing appliances adapted to the subject.

b. *Valves.*—Universally the separation of inspired air from expired air is accomplished by some type of valve. One of the simplest is the well known fluid valve of Müller described in 1859 (Tigerstedt(*f*)). Formerly they were much used filled either with water or mercury; but they offer considerable resistance to the air and have now been very generally displaced by valves of lighter construction. One form which has been widely used is the valve of Lovén consisting of two round brass boxes each enclosing a thin membrane of gold-beater's skin or condom rubber (Fig. 13). Small circular apertures suitably spaced and arranged in a circle round the peripheral attachment of the membrane serve for passage of air. The mechanics of this valve will be evident from the figure. Another favorite form is the metal valve of Thiry used by Tissot (Fig. 14). Boothby prefers the so-called flutter valve used in the most recent form of British and American army gas masks. He has devised a metal housing for the rubber flutter and finds the valve in this form perfectly competent. In case of doubt regarding the competency of a valve Boothby recommends the use of two valves one after the other in the inlet or outlet tubing (Boothby and Sandiford).

Fig. 12. The half mask as used by Boothby.

c. *Collecting Apparatus.*—The expired air can be collected either in a spirometer (Speck(*b*), Tissot), in a bag (Regnard, Douglas, C. G.), or

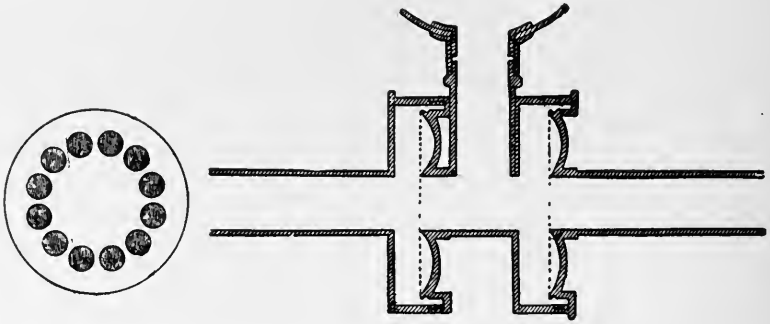


Fig. 13. Air valve of Lovén.

it may be measured by means of a gas meter and simultaneously sampled for analysis (Geppert(*a*)).

In the original spirometer method of Speck the inspired air was drawn from one spirometer and the expired air forced into another so that the difference in volume of inspired and expired air could be recorded and the

inspired air could also be readily measured at the same temperature and pressure preliminary to analysis. The bell of each spirometer was counterpoised and provision was made by mechanical means for compensating the increase or decrease in weight of the bell according as it was lifted from or depressed into the water jacket. The *Tissot method* as used in the French laboratories has been fully described by Carpenter(*a*). The spirometers are of special design and used principally in two sizes, one of 50 liters and another of 200 liter capacity. The height of the bell in the former is 60 cm. and the diameter 33; while in the 200 liter instrument the bell is 73 cm. high and 65 in diameter (Fig. 15). Air is admitted to the bell through a tube which terminates at the bottom of the spirometer in a 3-way stop-cock, A. The major portion of the weight of the spirometer bell is counterpoised by the weight R. The automatic adjustment of the counterpoise for the spirometer bell is accomplished by means of a water siphon. A glass cylinder, C, is made of such size that when

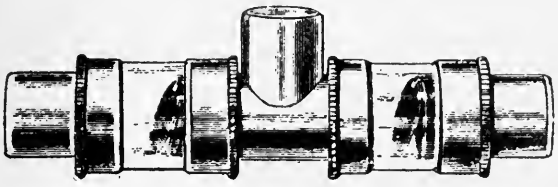


Fig. 14. Metal air valve of Thiry.

filled to the level of the spirometer the weight of the water in the cylinder exactly equals the increase in the weight of the spirometer bell due to its new position. When the bell rises or falls water is added to or taken from the cylinder C, by means of the siphon tube, D. Any increase or decrease in the weight of the bell due to the varying displacements of the volume of water by the mass of metal in the spirometer bell is thus exactly counterpoised by a like increase or decrease in the weight of water in the cylinder. The upright position of the counterpoised cylinder, C, is determined and maintained by means of two brass rods on which the cylinder travels. This siphon tube, D, is so arranged that it does not touch the cylinder, C, at any point.

A clinical form of spirometer or gasometer used by Boothby differs from the original form of Tissot in only minor features. A spirometer mounted on wheels as used in the Mayo clinic is illustrated in Fig. 16. The counterpoise of the bell in this instrument is hung over ball bearing wheels by means of steel piano wire. The main weight of the bell is balanced by a long hollow brass tube at the upper end of which are placed the necessary lead weights to counterbalance the bell exactly. The siphon arrangement of the original Tissot spirometer is used, but instead of drawing water from the gasometer itself to the counterposed cylinder, water is drawn from a special receptacle.

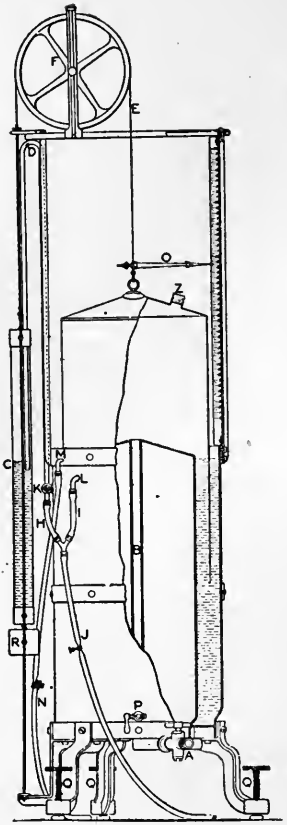


Fig. 15. Tissot Spirometer with capacity of 50 liters. *A*, three-way valve connecting air in bell of spirometer with outside air; *B*, tube leading to inside of bell; *C*, counterpoise tube compensating for change in weight of bell; *D*, siphon tube connecting *C* with water in tank; *E*, flat steel band supporting spirometer; *F*, wheel over which runs *E*; *H*, rubber tube connecting siphon tube with supply tube *J*; *I*, branch of supply-water tube leading to tank at *L*; *M*, *N*, overflow tube from tank; *O*, pointer; *P*, cock for emptying tank; *Q*, *Q*, leveling screws; *R*, lead counterpoise; *Z*, opening for gas sampling.

In this form of apparatus the scale for reading the volume of expired air is attached to the back side of the counterpoise tube.

In carrying out an experiment by the Tissot method the valves are first tested for tightness. Boothby carries out this test by filling the gas mask with water and letting it stand for a time for detection of leaks. A three-way valve at the side of the spirometer permits breathing from the subject into the room air or into the spirometer according to the position of the handle. The mask is attached securely to the face and the subject breathes for a time into the room air with the bell at its lowest position. The subject continues to breathe into the apparatus for a definite period of time, the inspired air being drawn through a pipe from outdoors. The valve is again turned at the end of an experiment. The temperature of the air is recorded by the thermometer in the top of the bell and a reading of the barometric pressure is taken.

With the Boothby apparatus several of the lead weights are slotted so that they may be readily removed. When all the weights are in place the bell is in perfect equilibrium at any point of its course, so that when the valve is open to the room air the bell will not change its position. When one or more of the lead weights are removed so that the bell is no longer perfectly counterpoised it will gradually drop. For the purpose of sampling this is a useful arrangement for the weight of the spirometer serves to drive expired air through the outlet tube, thus washing out room air from the main tube and the sampling connections. While the subject is breathing into the apparatus the extra weight of about 300 grams should be placed on the counterpoise so as to induce a slight negative pressure toward the spirometer. This serves to counterbalance the resistance which the air meets in the various tubes.

In the original *bag method of Regnard* the subject breathed through a Denayrouse mouth-piece and a pair of valves into a rubber sack of about 200 liters capacity. At the end of an observation a sample of about 150 c.c. of air was withdrawn for analysis and the balance of the contents was passed slowly through a series of absorbers and through a gas meter. In the Douglas method as originally described a mica or rubber-flap valve was used in connection with a mouth-piece and a tube of 20 mm. diameter led to a three-way valve of large bore which was connected with

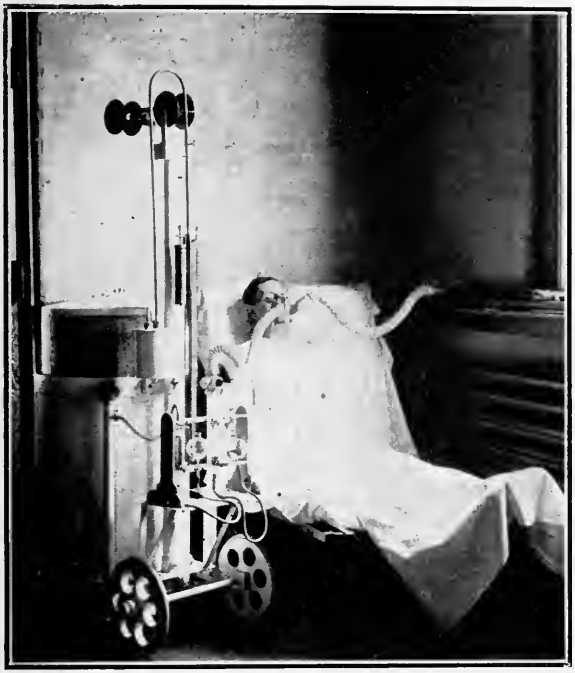


Fig. 16. Spirometer of Boothby and Sandiford as used in the writer's laboratory. Sampling tubes are shown on shelf above the wheels.

a wedge-shaped reservoir bag made of rubber-lined cloth (Fig. 17). This form of bag is more impervious than rubber and therefore more reliable. The shape of the bag permits it to be rolled up and emptied completely. The expired air is measured at the end of an observation by passing it through a meter and a sample is analyzed. By supporting the tube and valves on a light framework upon the head and resting the bag on another frame placed on the back the apparatus is made adaptable to a marching experiment.

It has proved especially valuable in mountain climbing (Haldane, Henderson, *et al.*) and other forms of open-air exercises. With violent exercise a bag holding 60 liters will not take the air expired during one

minute; but Krogh has shown that experiments of even much shorter duration are sufficient to give perfectly reliable results.

The *method of Zuntz and Geppert* of measuring the expired air as it is exhaled and collecting at the same time a continuous aliquot sample for analysis is an important one and has been very widely used in Europe. The subject breathes through a mouth-piece attached to a tee-tube connect-

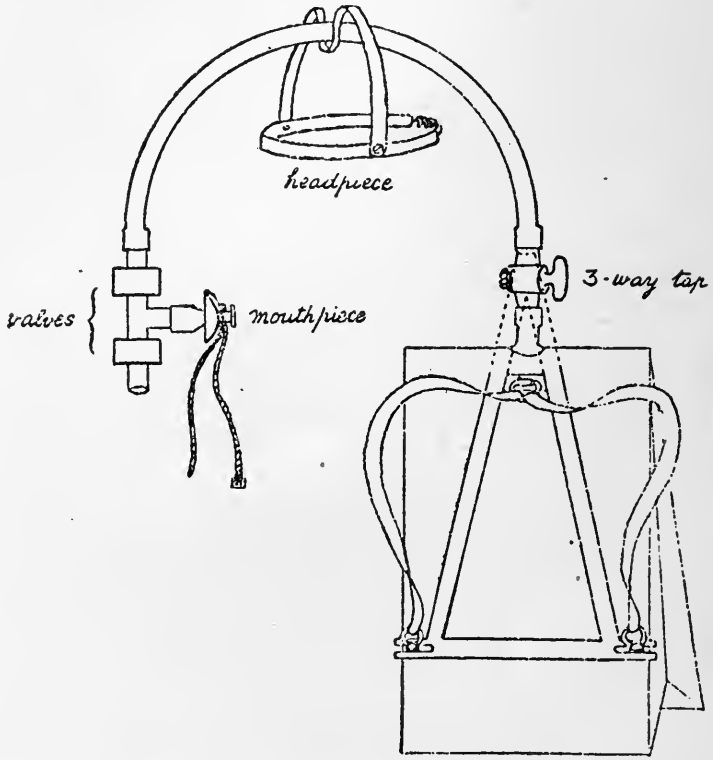


Fig. 17. Respiration apparatus of Douglas. The mouth-piece is of the Denayrouse type. The bag or bellows is provided with straps for carrying the apparatus on the back.

ing two valves (made of rubber and glass as used in the Zuntz laboratory, Magnus-Levy(*b*)) which separates inspired from expired air. The latter passes at once through a moist gas-meter. The continuous sample is taken over water by an automatic apparatus and is then immediately analyzed in a special analyzer in which the CO_2 is absorbed by potash and the oxygen by phosphorus. In the figure (Fig. 18) the meter is shown at the left and the special air analyzer is shown at the right. The expired air enters the apparatus at P. The sample is drawn through the narrow tube, L, by the lowering of the water-tube, H, which descends at a rate proportional to the ventilation as measured by the meter. As the tube, H, descends water

flows out at J and makes room for air in the two burettes (1) which fill from L at K and K. When these burettes are filled and contents measured the air is driven over, into the potash bulbs I, after which it is drawn back into the two burettes (2), where it is again measured. Thence it is passed into the phosphorus absorbers II and is finally measured for shrinkage due to loss of oxygen in the two burettes (3). The burette (4) is a "thermo-barometer" for recording any change in volume of the air contained in the

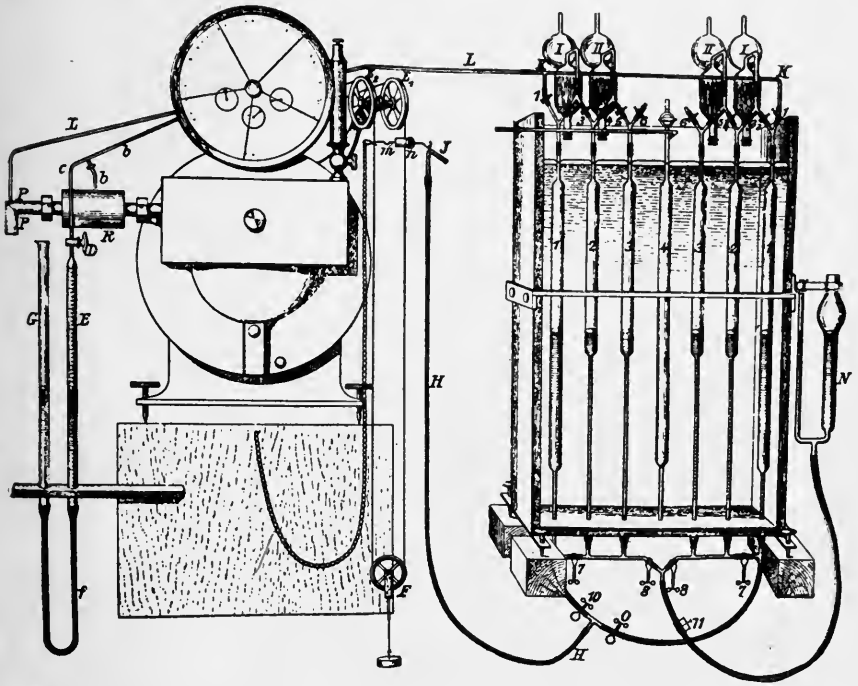


Fig. 18. Respiration apparatus of Zuntz and Geppert. The recording and sampling apparatus is shown at the left and the air analysis apparatus at the right. Air enters the apparatus from the lungs of the subject at *P*, a sample being drawn automatically through a tube *L*, and being passed in duplicate successively through the burettes numbered 1, 2 and 3. Burette 4 is for control. The part of the apparatus labeled *D*, *E*, *G* is a "thermo-barometer."

burettes due to alterations of temperature and pressure during an analysis.

The apparatus R. D. E. G. is another thermo-barometer for recording similar changes in the volume of the total ventilation. 100 c.c. dry air at 760 mm. pressure and 0° have been stored in two metal boxes one of which is inserted into the entrance tube of the gas meter at P, and the other into the exit tube. The air in these boxes communicates with the burette E. The enclosed volume of air will be affected by the temperature of the air entering and leaving the meter and by the atmospheric pressure, and the volume changes can be read off on the burette when the water in G and

E has been brought to the same level by moving G. The burette is so divided that, if a volume of say 107.4 is read off during an experiment, the volume of air which has passed through the meter can be reduced to normal conditions (0° and 760 mm. dry pressure) by multiplication with $\frac{100.}{107.4}$. This arrangement is certainly not more accurate and scarcely more convenient than to reduce by means of a table after reading the barometer and a thermometer placed in the exit tube of the gas meter.

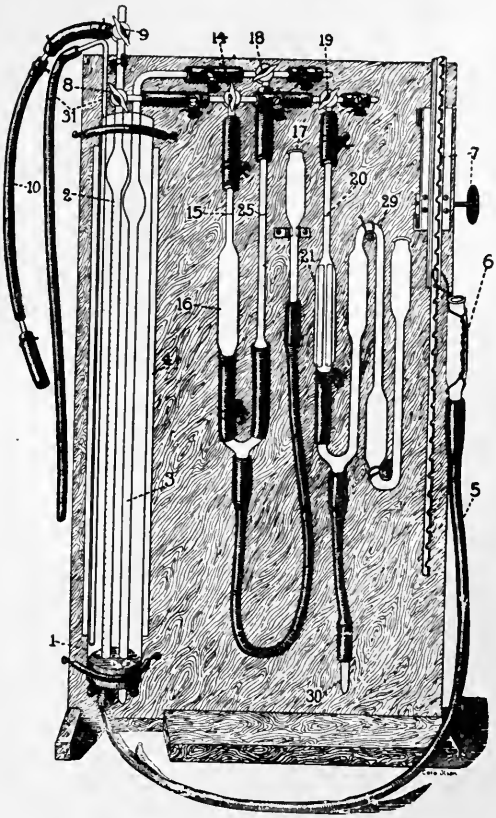


Fig. 19. The Haldane air analyser as used by Boothby. 1. Water-bath. 2. Burette. 3. Control tube. 4. Glazed glass back of water-bath. 5. Pressure tubing connecting burette and its mercury reservoir. 6. Mercury reservoir. 7. Ratchet and pinion. 8. Burette tap. 9. Sampling tap. 10. Sampling connection. 14. Potash tap. 15. Level marking on potash pipette. 16. Potash pipette. 17. Potash reservoir. 18. Control tube tap. 19. Pyro tap. 20. Level marking on pyro pipette. 21. Pyro pipette. 25. Level marking on manometer tube.

d. *Air Analyzers.*—With either the spirometer method or the bag method of collecting expired air or with the Jaquet type of chamber an absolutely essential part of the apparatus is a reliable device for determining carbon dioxid and oxygen volumetrically. The apparatus most used to-day is the Haldane analyzer. This apparatus is fully described by Haldane in his book entitled "Methods of Air Analysis." (Haldane(*c*).)

In a general way the method is as follows: A sample of air drawn into a 10 c.c. burette is accurately measured under the atmospheric pressure; the air is then passed into a potash bulb and back into the burette until a constant reading is obtained; the difference is the volume of CO_2 in the sample. In the same way the oxygen is absorbed in a solution of pyrogallol in strong potash and the difference in volume obtained represents the volume of oxygen in the sample.

As used by Boothby in the Mayo clinic the apparatus is shown in Fig. 19. Full details for manipulation of the apparatus and for calibration of

the burette are given in Boothby and Sandiford's book on "Basal Metabolic Rate Determinations."

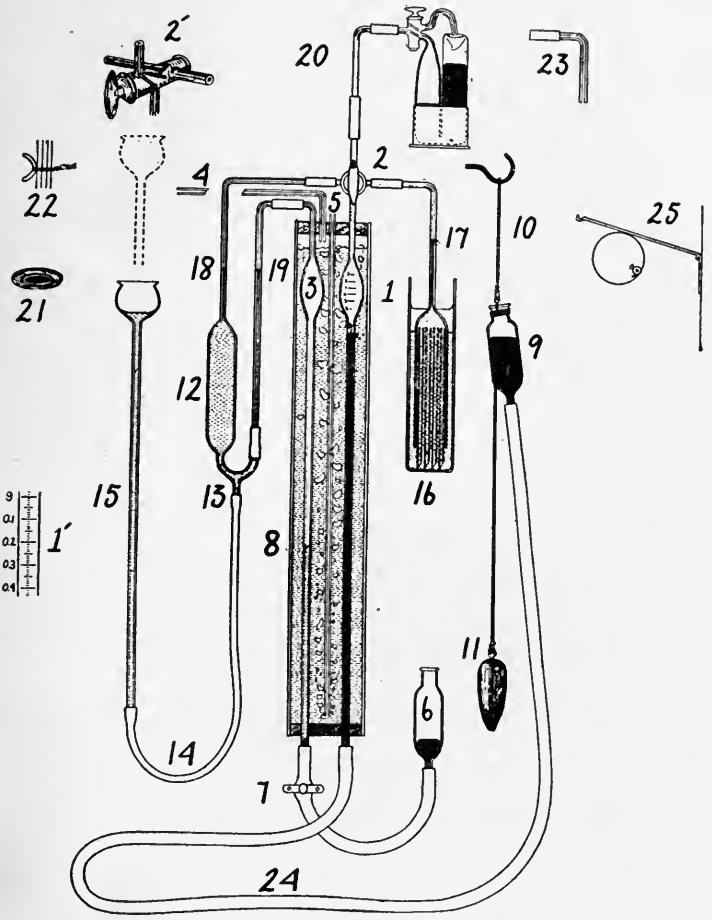


Fig. 19-a. Henderson modification of Haldane apparatus (Bailey). (1) Burette graduated in hundredths of a c.c.; (2) four-way stop cock at top of burette; (3) control tube same volume as burette; (4 and 5) glass tubes for circulation of air through water jacket; (6) mercury reservoir for varying pressure in control tube; (9) mercury reservoir for filling and emptying burette; (10 and 11) cord and counter-weight for suspending mercury reservoir; (12) potash pipette; (13, 14, 15) tubing and leveling bulb for potash pipette; (16) pyrogallol pipette; (17) leveling on pyrogallol pipette; (18 and 19) leveling marks on potash pipette; (20) connection to sampling bottle.

e. *Analysis of Outdoor Air.*—Haldane working with the portable form of his apparatus found that outside air contains 0.03 per cent of carbon dioxid and 20.93 per cent of oxygen. Benedict using the Sonden apparatus found as the result of 212 analyses in the Back Bay district

of Boston an average value of 0.031 per cent for carbon dioxid and 20.938 per cent for oxygen. In one series of 349 analyses nearly equally divided among 18 Haldane analyzers of the type described in Fig. 19 Boothby and Sandiford found the average CO_2 in the air taken upon the fire escape of their laboratory in the middle of Rochester, Minn., to be

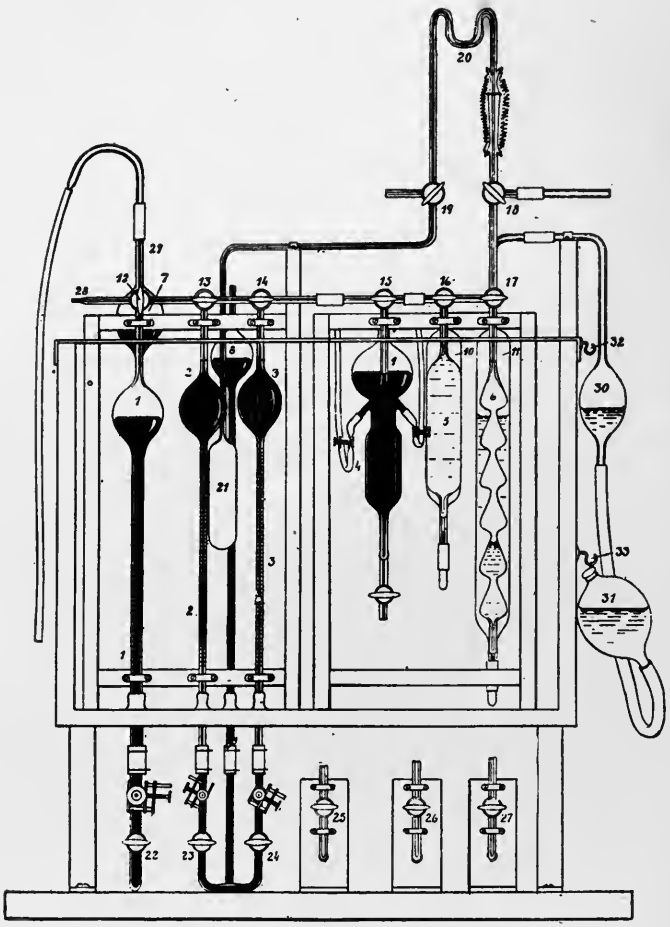


Fig. 20. The air analyser of Krogh. This apparatus like that of Zuntz and Geppert employs separate burettes for measurement of the air before and after absorption of CO_2 and oxygen. The air is moved from one burette to another by means of air pressure. For details of operation consult the original article.

0.037 per cent and the oxygen 20.930 per cent. In a second series of 343 analyses the average was 0.035 and 20.930 per cent. The higher percentage of CO_2 they ascribe to the fact that a large number of chimneys in the neighborhood of the laboratory gave out smoke which often drifted toward the laboratory.

Y. Henderson (Henderson and Morris) has devised a somewhat simpler form of the Haldane apparatus which has been improved in certain details by Bailey [1] at the N. Y. Post-Graduate Hospital. The degree of accuracy necessary for ordinary routine analyses for the determination of the basal metabolism in the hospital is easily attainable with this apparatus.

Krogh has recently described an apparatus which is accurate to 0.001 per cent. He finds that the sources of error which prevent the oxygen analyses from being highly accurate in the Haldane apparatus are intimately connected with the presence of water and dirt in the gas burette. Water must of course be present to insure the saturation of the gas with water vapor and dirt accumulates rapidly from the contact of mercury with the rubber tubing and with oxygen. Krogh gets rid of these interfering factors by employing three separate burettes (Fig. 20, 1, 2, 3) of which one (1) is employed exclusively for moving the air to and from the absorption pipettes, while the second (2) is of a suitable size for measuring the air before and after the absorption of CO_2, and the third (3) for measuring it after absorption of O_2. The water vapor necessary for saturating the sample air, when it has become partially dried in the absorption pipettes will be supplied by the first burette and the variations in the amount of water present has no influence upon the accuracy of the measurements. The two other burettes (2) and (3) contain just enough water to insure that the samples remain saturated.

A second improvement introduced by Krogh in this apparatus is that the mercury is raised and lowered in the burettes not by raising and lowering a mercury reservoir but by means of air pressure, an arrangement which obviates the use of rubber connections between the burettes and the reservoirs and besides facilitates the manipulation considerably (Krogh (*d*)).

Still another apparatus employing the open circuit method is deserving of mention. This is the apparatus of *Hanroit and Richet.* By means of air valves the inspired air and the expired air are separated, both being measured by meters. In addition the expired air is measured again after absorption of the carbon dioxid in potash. The first meter gives the volume of the inspired air, the second of the unchanged expired air, and the third the volume of the expired air minus the volume of carbon dioxid. The volume of inspired air less the final volume of expired air gives the amount of oxygen consumed. The method as carried out by Hanriot and Richet does not seem to be particularly accurate; but Krogh expresses the opinion that the method has great possibilities if used with modern gas meters of sufficient size and placed in a water bath where the volumes measured would be subject to the same fluctuations. Krogh notes that

[1] This construction of the apparatus is made by E. Machlett & Son, 153 East 84th Street, New York City.

the volume recorded by a meter is independent of the rate only within certain limits corresponding roughly to 100 complete revolutions per hour (Krogh(*c*)). As Benedict has shown the volumes recorded at higher rates than this are smaller than the actual volumes, but if the high rate is constant and the meter is calibrated at such a rate it is quite possible to record volumes with no appreciable error. In such a method as that of Hanriot and Richet the meter employed for measuring the respiration of a man at rest should be capable of measuring correctly not less than 12 meters per revolution, and since in heavy muscular work the total ventilation may be multiplied tenfold over that of the resting rate of respiration a meter for measuring the ventilation of the lungs would need to have a capacity of 120 meters. Krogh has recently devised a spirometer for calibrating gas meters which should simplify this process and render the use of gas meters much more reliable. In the paper describing this apparatus Krogh notes that in wet meters with a constant quantity of water the volume per revolution increases with increasing rate but can be determined with sufficient accuracy. Dry gas meters he finds are much less accurate than wet test meters.

2. Closed Circuit Instruments.—There are two well-known forms of respiration apparatus used with mouth-pieces or nose-pieces and constructed on the closed-circuit principle. The first of these is the so-called Universal respiration apparatus of Benedict(*d*)(*e*); and the second is a modification of the instrument constructed by Haldane and Douglas devised by Krogh(*a*). To speak of the second form first, Krogh has so devised his instrument that it may be used continuously for a considerable period of time by a man at rest. The soda lime absorber is capable of retaining 1000 liters of carbon dioxid. Oxygen is admitted from a cylinder, being passed through a meter which records electrically by closing a circuit each time the meter revolves once and has, therefore, passed a certain volume of oxygen. A recording spirometer gives a quantitative record of the respiratory movements. Only oxygen absorption is determined as the apparatus is usually employed, but carbon dioxid determinations can be made by drawing samples of inspired and expired air from certain parts of the apparatus. So far as known to the writer this form of apparatus has never been used in the United States.

The apparatus of Benedict on the other hand has been used quite extensively. The writer has made almost continuous use of one of these over a period of nearly twelve years. It has been modified and improved from time to time and is used to-day as shown in Fig. 21. Attachment to the respiratory passages of the subject is effected by means of the Denayrouse mouth-piece or the rubber nose-pieces of Benedict. Quite recently also the half mask of Boothby has been adapted to this use and has given much satisfaction. It is far more comfortable than either the mouth-piece or the nose-piece. The apparatus is constructed with three trains

of absorbers. The first immediately following the rotary blower consists of two Williams bottles containing sulphuric acid which wash out all of the water from the expired air and water left over from the moistener bottle. The other two are duplicate trains for absorption of carbon dioxid. Each consists of two soda lime bottles and a Williams bottle containing sulphuric acid. By thus reducing the size of each unit a smaller and much less expensive balance can be employed for weighing the absorption of

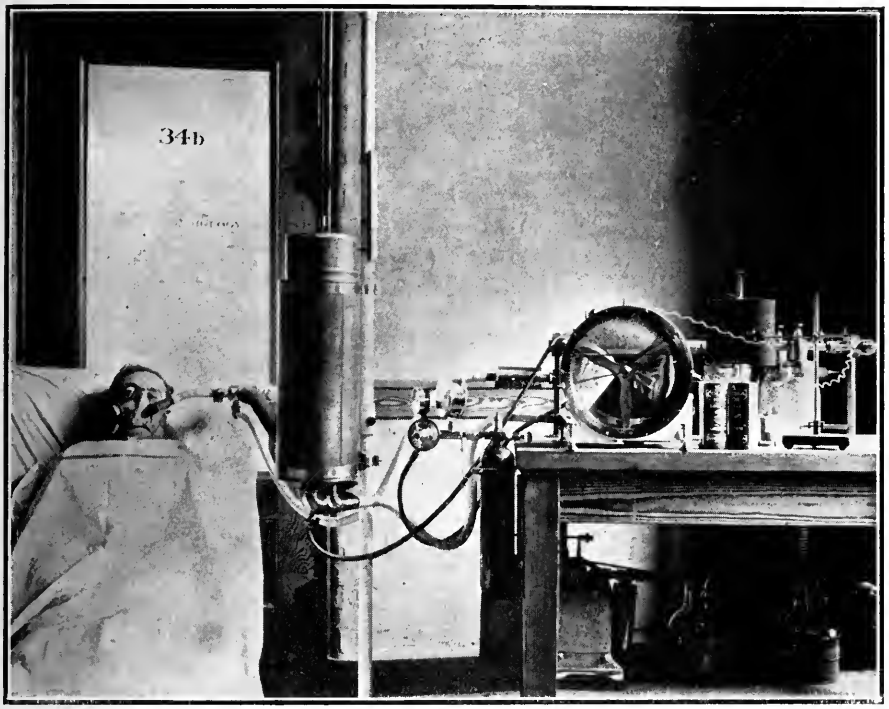


Fig. 21. The Benedict Universal respiration apparatus as employed by the writer. The spirometer and tubes leading to the face mask are carried on a separate stand so that they may be adjusted to a subject in the reclining, sitting or standing position. Oxygen is supplied from a pressure cylinder and is measured on its way to the spirometer by the meter. Two sets of absorbers are used so that observations may be made continuously in successive periods.

carbon dioxid. Oxygen is fed into the circuit from a high pressure tank through a reduction valve and on its way is measured by a Bohr meter. The spirometer and tubes leading to the subject are mounted on a separate standard so that the height of the mouth-piece can be adjusted for a subject in the reclining, sitting or standing position. The same apparatus, therefore, can be used for basal metabolism, for work experiments, or for observations on the influence of food.

The technique as worked out in the writer's laboratory for operation of this instrument is briefly as follows. Let us suppose a basal metabolism

is to be determined. The subject comes to the laboratory early in the morning after having taken a very light breakfast of black coffee and toast, or no breakfast at all. For half an hour the subject is required to lie perfectly still wearing the nose clip and breathing through the mouthpiece into the room air or breathing through the face mask into the room air. He thus becomes accustomed to all the sensations incident to the experiment. A slight pulsation of the air current transmitted from the blower is felt by the patient unless special means is taken to muffle it. Such vibrations may become very annoying to the subject.

When the absorbers have been weighed and the patient has become sufficiently composed the blower is started and the apparatus is run idle blowing the air round and round through the circuit for at least two minutes in order to make certain that any carbon dioxid left over from a previous observation shall have been completely removed. With a small weight placed upon the spirometer this preliminary run serves also to test the entire circuit for tightness. If after a minute or two the spirometer holds its level the entire circuit is air tight and the experiment may proceed. The oxygen meter is read at this point.

With an intelligent subject it is our custom to let the subject turn the valve himself, instructing him to do so just before beginning an inspiration. With a subject wholly unaccustomed to the apparatus or not sufficiently intelligent to understand what is meant by "respiratory pause" the observer quickly turns the valve at the moment of respiratory rest intervening between the end of an expiration and the beginning of an inspiration. In either case the second hand of a watch is read at the instant the valve is thrown. If the air current is passed through a moistening bottle which follows the acid absorber in the carbon dioxid train the air comes to the subject feeling rather soft with moisture, and also feeling perhaps a little cool from the temperature of the water. These are the only sensations which the subject should experience, when the valve is thrown connecting him with the circuit. There should be no trace of irritation either from the air itself or from the apparatus connecting with his face.

With a little experience oxygen can be fed in through the meter at approximately the rate at which it is absorbed by the subject. This method is preferable in the writer's opinion to the intermittent feeding of oxygen, providing only that the rate of flow be kept low enough so that at the termination of the observational period the spirometer shall be lower than it was at the moment the valve was first thrown. It is far more important to terminate the observation correctly with reference to the phase of respiration when the valve is thrown than it is to terminate the observation on the second by the watch. The observer, therefore, gives his entire attention to throwing the valve and only notices the position of the second hand after he has successfully thrown the valve. The blower is allowed

to continue running for two or three minutes until the spirometer ceases to fall and oxygen is then admitted until the spirometer comes back to the original level. The blower continues running for a few seconds longer to make certain that this level will be maintained, the oxygen now having been stopped, whereupon the current is turned off stopping the blower. The oxygen meter is now read.

If a second observation is to follow immediately the valves are thrown connecting with the second set of absorbers and the blower immediately started. As soon as it is certain that the second train of absorbers is air tight the second period can be started. The absorbers of the first train can be weighed while the second period is running. The barometer is read and the temperature of the water meter measuring the oxygen is recorded. The volume of oxygen is then reduced to 0° and 760 mm., and the carbon dioxid obtained in grams is likewise reduced to the standard conditions. The respiratory quotient is obtained by division of the volume of carbon dioxid by the volume of oxygen.

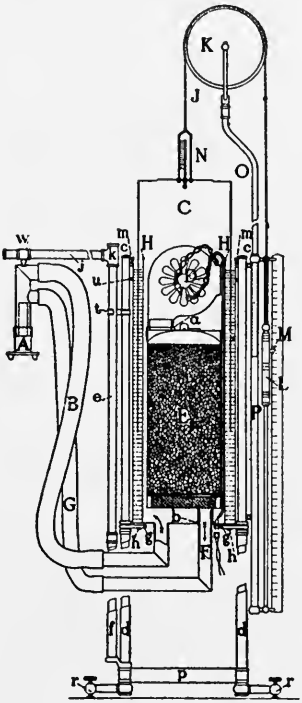


Fig. 22. Portable respiration apparatus of Benedict and Collins. *A*, mouthpiece; *B*, tube conducting expired air to bell *C*; *D*, hair dryer; *E*, soda-lime container; *F* and *G*, tubes conveying air current to mouthpiece *A*; *III*, tank in which bell *C* floats; *J* and *K*, cord and pulley supporting bell *C*; *L*, counterpoise; *M*, pointer on counterpoise; *N*, thermometer; *O* and *P*, supports for pulley *K*. *a*, rubber gasket; *b*, rubber gasket; *c*, *c*, tubes supporting spirometer; *d*, *d*, lower part of frame supporting spirometer; *e* and *f*, telescoping tubes supporting mouthpiece and tubing; *g*, *g*, supporting plates; *h*, *h*, knobs fitting into *g*, *g*; *jk*, part of support for mouthpiece and tubing; *mm*, attachment to support *c*, *c*, to tank *III*; *p*, circular band connecting four tubes, *d*, *d*; *r*, *r*, leveling screws; *t*, sliding ring; *u*, knobs for support of apparatus when collapsed; *w*, sliding ring.

Recently several forms of so-called portable instruments constructed on the general principle of the universal respiration machine of Benedict have made their appearance. The best of these doubtless is the one described by Benedict and Collins. It may be doubted, however, whether it is wise to attempt to make the determination of basal metabolism a bedside or office procedure. Special

laboratories for this purpose in hospitals or elsewhere will continue to give more accurate results, as is true of x-ray and electrocardiographic work and for the same reasons.

III. Methods of Calculating the Heat Production from the Respiratory Exchange

Historically four distinct methods (LeFèvre(*g*)) have been employed for the calculation of the heat production from the chemical changes going on in the body. In each case the method rests upon the fact established by Lavoisier that the products of respiration are the products of combustion.

1. Calculation from Heats of Combustion of Carbon and Hydrogen.—This method possesses only historical interest to-day, yet it should be presented briefly for the sake of the underlying principle involved. In 1783 Lavoisier published a celebrated work upon the respiratory metabolism and calorimetry of the guinea pig. The chamber in which the animal was contained was traversed by a current of air from which the carbon dioxid was absorbed at the entrance and exit in potash bottles. The gain in weight of the latter less the gain in weight of the former gave the carbon dioxid produced by the animal. In ten hours a guinea pig gave off 3.33 gm. of carbon, which from previous experiments Lavoisier knew was equivalent in heat value to 326.76 gm. of ice melted at 0°. He proved this by placing the pig in an ice calorimeter and found 341.08 gm. melted.

In 1785 Lavoisier, applying his work to the human subject as well as to the animal, established the fact that out of 100 parts of oxygen absorbed, 81 parts only reappeared as carbonic acid gas; and he concluded that the other 19 parts were combined with hydrogen to form water (Gavarret). Respiration was thus seen to be accompanied by double combustion and Lavoisier proposed by quantitative studies of the respiration to determine the proportion in which oxygen is partitioned between carbon and hydrogen of the materials in the blood to produce carbonic acid gas, water and heat.

But this is not all. With Séguin, Lavoisier (Lavoisier and Séguin(*b*)) made a series of experiments upon the human subject and demonstrated that carbon dioxid is produced and oxygen is absorbed in proportion to the mechanical work effected by the organism. "By this new discovery Lavoisier raised the theory of combustion to the level of a great generalization and revealed for the first time the essential source of all animal energy" (LeFèvre(*g*)).

A method devised by Dulong consisted simply in measuring directly the CO_2 produced and indirectly the water by assigning to hydrogen all the oxygen which was not recovered as CO_2. Since, however, it is not certain that all of the oxygen which escapes combination with carbon serves

only for the formation of water, Boussingault(*b*) sought to establish the exact amount of hydrogen burned by striking an exact and complete balance of materials between the ingesta and the ejecta of the body.

The heat of combustion of carbon and hydrogen having already been established at 8.040 and 34.46 kilo-calories per gram respectively, Helmholtz calculated by Dulong's method that a man of 82 kg., giving off in the respiration in 24 hours 878.4 gm. CO_2 or 239.6 gm. C produced ($239.6 \times 8.04 =$) 1,925 calories. The excess of oxygen going to form water combined with 13.615 gm. H producing ($13.615 \times 34.46 =$) 469.172 calories. The total heat production therefore was 2395.55 Cal.

Vierordt by a method entirely analogous to that of Boussingault calculated the heat production from the known metabolism of food as follows: Taking the average ration of the adult at 120 gm. protein, 90 gm. fat and 340 gm. carbohydrate and leaving out of account the hydrogen of the carbohydrate, because it was known to be saturated with oxygen, there were in

		C	H
120	gm. protein	64.18	8.60
90	" fat	70.32	10.26
340	" carbohydrate	146.80	
	Total	281.20	18.86

But the urine and feces contained unoxidized carbon and hydrogen determined at 29.8 gm. for the former and 6.3 gm. for the latter. The net combustion, therefore, was ($281.20 - 29.8 =$) 251.4 gm. C and ($18.86 - 6.3 =$) 12.56 gm. H, and the heat production

$251.4 \times 8.04 =$	2031.31	Cal.
$12.56 \times 34.36 =$	332.82	"
Total	2364.13	"

These methods of calculating the heat production upon the heats of combustion of hydrogen and carbon contained in the food as if the hydrogen and carbon were free gases are now known to contain an error of at least 11 or 12 per cent. The heat of combustion of formic acid (CO_2H_2), for example, is not equal to the combustion heat value of C and H_2; for the heat value of H_2 is 683 Cal. per gram-mol and of C is 943 Cal. per gram-mol; whereas that of CO_2H_2 is only 694 Cal. per gram-mol. The difference between the combustion heat value of CO_2H_2 and the sum of the values for C and H_2 is called the heat of formation.

The heat production, therefore, must be based upon the combustion of the organic foodstuffs themselves.

2. Calculation from the Heats of Combustion of the Organic Foodstuffs.—Berthelot and André determined the physiological heat value of protein (egg albumin coagulated and dried at 100° C.) by burning in

the calorimeter and deducting the quantity of heat represented by the urea formed from it. The bomb value of the protein was 5.690 calories and the urea 833, leaving a net value to the organism of 4.857 calories per gram. The average values for eleven different food proteins was found by them to be 5.691 Cal. and the net value after deducting the urea formed was 4.750 Cal. per gram.

In the conclusion to their paper Berthelot and André state that the influence of the intestinal excretions "cannot modify these figures very much for the feces in man form a very small fraction of the weight of the food." The unabsorbed residue from proteins it is now known, however, constitute as much as 10 to 15 per cent of the food; hence they are by no means negligible.

The exact physiological heat values of these organic foodstuffs was first resolved with a high degree of exactness by Rubner(*d*). He proceeded from the known fact that in the case of proteins, urea is not the only nitrogenous waste product and that some of the others have very different heat values from that of urea. Besides he saw the necessity of deducting the heat value of the feces resulting from the food in question. An example of the method employed by Rubner may be given as follows:

Lean meat free of connective tissue was taken and dried; it was then macerated in alcohol to insure its complete dehydration. After drying again and evaporation of the alcohol it was macerated once more in ether. The albumin resulting had the appearance of *papier maché* and was practically free of salts. When this material was powdered and burned a bomb heat value of 5.754 Cal. per gram was obtained.

A dog was fed for eight days with 116.8 grams of the dried and purified protein daily. The urine for the first six days was rejected, and that for the 7th and 8th days only saved, the dried residue of which gave a heat value of 2.706 calories per gram. The heat value of urea he found to be only 2.523 Cal. or 7 per cent less than that of the whole urine. One gram of the dried matter was found to contain 0.414 gm. of N, from which it was found that 1 gm. of N in the urine represented 6.690 calories.

The feces contained 37.8 gm. of dry matter daily. The loss by non-absorption therefore was 3.24 per cent. Burned in the calorimeter this dry matter was found to contain 5.722 calories per gram. When the ash was deducted it was found to have a heat value per gram of 6.852 calories, and the nitrogen was found to be 7.02 per cent. The net physiological heat value therefore could be calculated as follows:

Ingested 100 gm. dry protein of meat 575.40 Cal.

Excreta { Urine—109.450 Cal.
Feces— 18.540 "

Total — approx. — 128.000

Difference — — 447.400 or 4.47 Cal. per gram.

Making further corrections for the heat of imbibition and of solution this figure in the particular experiment cited was reduced to 4.42 Cal. which was 76.8 per cent of the gross heat value of the protein as fed.

Since 100 grams of the dried albumin of meat contained 16.59 gm. of N and its combustion gave a heat value of 4.424 Cal. per gram each gram of N had a heat value of 26.66 Cal.

With unwashed meat the value came out 25.98 calories per gram. In the same research Rubner(*d*) calculated that the body protein of a starving rabbit had a physiological heat value of 3.842 Cal. per gram, or 71.9 per cent of its gross heat value, or again 24.94 calories per gram of N.

The mean physiological heat value for a number of animal proteins—paraglobulin (4.371), egg albumin (4.307), casein (4.404), fibrin (4.179)—was found to be 4.21 Cal. per gram. Conglutin was taken as a type of vegetable protein and was found to have a value of 3.97 calories.

Since out of 100 grams of mixed protein in human food about 60 per cent is taken from animal sources and 40 per cent from vegetable, Rubner calculated the mean value for food protein in general at 4.11 Cal. per gram.

Accepting the bomb values of Stohmann for carbohydrates and considering the preponderance of starch in human dietaries Rubner estimated the physiological heat value of carbohydrates in general (making deduction of cellulose) at 4.1 Cal. per gram. For fat he adopted the mean value of 9.3 Cal.

These values—Proteins—4.1 Cal.
Fat — 9.3 "
C. H. — 4.1 " have become standard in the literature of metabolism and are now generally used.

Atwater and his collaborators in this country have adopted a somewhat different method of arriving at the physiological heat value of the foodstuffs. He lays down the principle that the combustible value to the body is found by subtracting from the heat of combustion of the *utilizable* food the heat value of the urine corresponding to the food in question. The average utilization (i. e., ingestion less feces) of the several classes of foods he gives as follows (Atwater, Benedict, Smith and Bryant):

	Prot.	Fat	C. H.
Animal Foods	97%	95%	98%
Cereals	85	90	98
Legumes dry	78	90	97
Sugar and Starch	..	..	98
Legumes, fresh	83	90	95
Fruits	85	90	90

The fats and carbohydrates being completely burned in the body, the heat value to the body is equal to the total calorimetric value of the portion ab-

sorbed. The total heat value of the urine arising from the incomplete oxidation of proteins, its heat value represents that fraction of the potential energy of the proteins *absorbed* which the body does not utilize. Utilization thus is used in two senses. From the standpoint of absorption it is that part of the food which exceeds the amount excreted through the bowel. From the standpoint of energy it is that part of the *absorbed* food diminished by the potential energy of the bodies excreted in the urine. Comparing the method of Rubner with that of Atwater, it is seen that in the former calorimetric heat value equals heat of the specific food ingested less the heat of the feces less heat value of the urine. According to Atwater the calorimetric heat value equals the heat value of the utilizable food less heat value of the urine.

The method of Rubner is more direct and thermochemically is more correct; but it is impracticable in its application to man for it requires the ingestion of a perfectly pure (salt free) foodstuff. The method of Atwater is open to the objection that he assumes the same heat value for the proteins of the feces as for the corresponding food protein. It has the advantage of simplicity, however, in that it employs a coefficient of utilization and can be used for a mixed diet both in animals and man.

Woods made 56 determinations of the heat value of the urine in Atwater's laboratory and found an average value per gram of N of 7.9 Cal. If this 1 gram of N represents 6.25 gm. of protein destroyed, for each gram of protein absorbed and burned there is a loss of $(7.9 \div 6.25 =)$ 1.25 Cal.

The heat value of a food protein may then be found by the following method. Protein of meat has (table above) a utilization of 97 per cent. Its heat value is 5.65 Cal. The energy of the portion utilized is $5.65 \times 0.97 = 5.48$ Cal. per gram. But from this value must be deducted the heat value of the urine, which according to Wood's determination is $1.25 \times 0.97 = 1.20$ Cal. The physiological heat value of meat for the human subject, therefore, is $(5.48 - 1.20$ Cal. $=)$ 4.28 or in round numbers 4.25 Cal.

The bomb heat value of *cereal protein* Atwater found to be 5.8 Cal. per gram; its utilization was 85 per cent; therefore, its physiological heat value would be $(5.8 \times 0.85) - (1.25 \times 0.85) = 3.87$ Cal. per gram. The mean physiological heat value for all animal proteins was given by Atwater at 4.27 Cal. and that of all vegetable proteins at 3.74 Cal. or 4.05 Cal. per gram for food proteins generally. It is now known, however, that the utilization of cereal protein such as that of bread is more commonly 92 per cent rather than 85 per cent as found by Atwater. This would change his figure for vegetable protein from 3.74 to 3.98 Cal. per gram, and if the percentage of animal and vegetable proteins in the diet be placed at 40 and 60 which more nearly accords with practice in most

countries outside of the United States the mean heat value to the body would be: $\frac{4.27 \times 40 + 3.98 \times 60}{100} = 4.100$ Cal. which is the average value given by Rubner.

The physiological heat values of fat and carbohydrate are found by the Atwater method in the same manner except that no deduction is made for the urine. The average utilization in the human subject for animal fat being 95 per cent and for vegetable fat 90 per cent, and the bomb values being 9.5 Cal. and 9.4 Cal. respectively, the value to the body is 9.02 and 8.46 Cal. for the two or 8.75 Cal. for food fats in general. For carbohydrates the factors are 4.2 Cal. per gram bomb value, and 98 per cent utilization. Therefore, the value to the body is 4.1 Cal.

Both Rubner and Atwater have justified the heat values of the several foodstuffs to the body by direct calorimetric experiments upon the dog and man respectively. Rubner(*f*) hit upon a very clever method of confirming his heat values with the aid of his calorimeter. In one experiment he fed a dog a large amount of protein and a small amount of fat; in another just the reverse. The metabolism was as follows:

1st Exp.	N elim.	10.09 gm.
	C. of fat oxidized	9.06 "
	Total Calories	379.50 Cal.
2nd Exp.	N. elim.	2.95 gm.
	C. of fat	19.12 "
	Total Calories	311.0 Cal.

Let x be the heat value of a gram of nitrogen and y the heat value of a gram of C from fat. Then, $10.09x + 9.06y = 379.5$ Cal.

$2.95x + 19.12y = 311.0$ "

From which $x = 26.70$ Cal.

$y = 12.15$ "

Now 1 gram of N corresponds to 6.49 grams pure protein of meat—hence 1 gm. $= \frac{26.70}{6.49} = 4.05$ Cal. One gram C corresponds to 1.3 gm. pure fat; hence 1 gm. $= \frac{12.15}{1.3}$ Cal. $= 9.31$ Cal.

Atwater in a series of 27 studies on human subjects, 14 of which were carried out in the calorimeter devised by Rosa, found a difference between the direct measurement of heat eliminated and the theoretical heat production as calculated from his factors of less than 1 per cent, which may be taken as satisfactory proof that these values for the human subject are substantially correct. [2]

[2] The only difference of any consequence between Rubner's and Atwater's values applies to fat. Modern authorities who have been most under the influence of the

The method of *Alimentary Calorimetry* consists then simply of finding the average daily ingestion in terms of protein, fat and carbohydrate and multiplying by the standard physiological heat values. Thus Gautier(*b*) gives the average dietary of a middle class Parisian as 102 grams protein, 56 grams fat and 400 grams carbohydrate. His average energy utilization, therefore, would be: $102 \times 4.1 + 56 \times 9.0 + 400 \times 4.1 = 2562$ Calories. If a person on this diet were in equilibrium of nitrogen and weight, his energy production would be equal to this sum; otherwise not. Besides, weight is not a satisfactory criterion of energy equilibrium and the utilization when the diet is made up of different articles will vary considerably. All we are justified in saying, therefore, is that an average regimen of this sort represents such and such an energy value to the body. Some persons would gain in weight on it; others would lose. Another example is the following taken from the nutritional surveys of Army Camps in the United States made by the Medical Department of the Army in 1918 (Murlin and Miller).

TABLE 1

NUTRIENTS AND ENERGY CONSUMED IN TRAINING CAMPS OF U. S. ARMY

	Food per Man per Day				Consumed Distr. of Fuel Value
	Nutrients	Supplied	Wasted	Consumed	
Averages 427 messes	Proteins gm...	131	9	122	14%
	Fat gm.....	134	11	123	31%
	Carbohydrate .	516	31	485	55%
	Fuel Value, Cal.	3899	266	3633	100%

The "Fuel value consumed" in this and similar tables gives the energy value to the body of the food consumed and not the amount of energy released by the body. Upon the diet of the Army Camps in 1918, the average recruit gained nearly six pounds in weight during a period of five months training, showing that the energy content of the food was considerably more than sufficient to sustain the muscular activity of hard training and to maintain body weight. [3]

The *actual heat production* in any given case can be computed from the physiological heat values just discussed provided the output of carbon and nitrogen can be determined, and provided it be assumed that all of the carbohydrate fed is burned before fat burns. This method of calculation

German school of metabolism have adopted Rubner's values of 9.3; while French authorities like Gautier and LeFèvre have accepted the work of Atwater as equally conclusive with that of Rubner and have adopted a mean value between the two authorities of 9.0 Cal. per gram. Since the methods of calculating the actual heat production by use of these values have been largely superseded by the method of thermal quotients to be described in the next section, the controversy over these values has subsided.

[3] Recruits fed in this way for several months have almost certainly a higher basal metabolism (see page 607) than civilians of the same initial weight and age, and it is not yet certain that the benefit from the standpoint of muscular efficiency is commensurate with the cost in superfluous metabolism. This is a problem which requires careful study by the army itself.

was first applied by Rubner to the results obtained by Voit and Pettenkoffer on a fasting man (Lusk(*h*)). These observers had found that their subject, weighing 71.09 kgm., gave off in the respiration and in the urine 207.11 gm. carbon and in the urine 11.33 gm. nitrogen. Deducting from the total carbon the carbon (3.28 times the N) belonging to protein the remainder was calculated as carbon of fat and it was learned that the man had burned 70.81 gm. protein and 22.1 gm. fat. Rubner applied his physiological heat values for a gram of N in starvation (24.98 Cal.) and for a gram of carbon in fat (12.3 Cal.) and learned that the total energy production of the man in twenty-four hours was:

11.33 gm. N x 24.98 =	283 Cal.
166.95 gm. C of fat x 12.3 =	2091 Cal.
Total	2374 Cal.

When the food contains only fat and protein exactly the same method is used for calculating the heat production, except that the heat value of nitrogen in the urine has a different value (see page 552). When the food contains carbohydrate any gain or loss of C to the body may be estimated as fat, it being assumed that the amount of glycogen in the tissues is the same at the end of an experiment as at the beginning. It will be seen later that Rubner, employing this method of calculation in experiments on the dogs whose heat production was measured simultaneously in a calorimeter, found perfect agreement between the heat as calculated and as measured, thereby proving the essential correctness of the method. Atwater's method of calculation in similar experiments on human subjects was different, but proved to be equally correct.

3. The Method of Thermal Quotients of O_2 and CO_2.—When an organic foodstuff is burned in the animal body a definite amount of oxygen is absorbed and a definite amount of CO_2 is formed and eliminated. If the heat formed by such a combustion is known the heat value of a gram of oxygen absorbed or of a gram of CO_2 eliminated may be expressed as a simple quotient of heat divided by the weight of the gas. Since the measurement of the respiratory gases by volume is an easy matter the thermal quotient can be expressed also in relation to a liter of gas at 0° C. and 760 mm. of pressure or at any other desired temperature.

a. *Calculation of Thermal Quotients.*—If we suppose that protein burns only to the stage of urea the thermal quotient for this foodstuff may be calculated from the following equation:

$$\underset{\text{Albumin}}{C_{72}H_{112}N_{18}SO_{22}} + 77\ O_2 = 63\ CO_2 + 37\ H_2O + 9\ \underset{\text{Urea}}{CON_2H_4} + H_2SO_4$$

According to this equation 1.612 gm. of protein yielding 7.810 Cal. of heat would consume 77 molecules of O_2 weighing ($77 \times 32 =$) 2.464 gm. and

63 CO_2 weighing (63 × 44 =) 2.772 gm. For oxygen the thermal quotient would be (7.810 ÷ 2.464 =) 3.19 Cal. per gram and for CO_2 (7.810 ÷ 2.772 =) 2.82 Cal. per gm. Or, on the basis of volume at 0° and 760,

4.54 Cal. per liter of O_2
and 5.44 Cal. per liter of CO_2

For fat the thermal quotient may be calculated from the following equation: $C_{57}H_{104}O_6 + 80.O_2 = 57\ CO_2 + 52\ H_2O$
Triolein

From this it follows that 0.884 gm. of this particular fat yielding 8.423 Cal. would require 80 molecules of O_2 weighing (80 x 32 =) 2.560 gms. and 57 molecules of CO_2 weighing (57 x 44 =) 2.508 gms. One gram of O_2 therefore has a heat value of (8.423 ÷ 2.560 =) 3.29 Cal. and one gram of CO_2 (8.423 ÷ 2.508 =) 3.35 Cal. or, on the basis of volume at 0° and 760,

4.70 Cal. per liter of O_2
and 6.58 Cal. per liter of CO_2

For carbohydrate the equation is: $C_6H_{10}O_5 + 6\ O_2 = 6\ CO_2 + 5H_2O$ and the thermal quotients are: 5.09 Cal. per liter of O_2
and 5.09 Cal. per liter of CO_2
The results may be summarized as in the table below.

TABLE 2

THERMAL QUOTIENTS (LEFÈVRE(*g*))

	Cal. per Gram		Cal. per Liter at 0° and 760 mm.		at 18° C.		Gms. O_2 Consumed per Gram of Foodstuffs Burned
	O_2	CO_2	O_2	CO_2	O_2	CO_2	
Proteins	3.19	2.82	4.54	5.44	4.261	5.104	1.524
Fats	3.29	3.35	4.70	6.58	4.410	6.174	2.896
Carbohydrates.	3.56	2.59	5.09	5.09	4.776	4.776	1.185

To estimate the mean thermal quotient for a mixed diet the method is a simple one. For example, take the mean food consumption of the average soldier in the training camps (p. 554) namely, 122 gm. protein, 123 gm. fat and 485 gm. carbohydrate. The amount of oxygen required for the combustion of these quantities of the several foodstuffs would be:

122 gm. Protein	x 1.524 =	185.9 gm.	O_2
123 gm. Fat	x 2.896 =	356.2 "	"
485 gm. C. H.	x 1.185 =	574.7 "	"
	Total	1116.8 "	"

Multiplying each of these quantities of oxygen by the respective thermal quotients (see table above) for the different foodstuffs:

	185.9	gm.	$O_2 \times 3.19 =$	593.1	Cal.
	356.2	"	" $\times 3.29 =$	1172.0	"
	574.7	"	" $\times 3.56 =$	2046.0	"
Sums	1116.8	"	" $=$	3811.1	"

From this calculation 1 gm. $O_2 = 3.41$ calories.

For a liter of oxygen at 18° C. the mean thermal quotient would be 4.60[1] Cal. (nearly).

Laulanié(*a*) conducted experiments on small animals at or near this temperature by means of a small calorimeter and computed the oxygen absorbed by analysis of the air of the chamber after a short period of confinement. The average value of the thermal quotient found by him was 4.71 Cal. per liter as calculated from the metabolism and 4.75 Cal. as measured by the calorimeter.

Atwater and Benedict(*c*) in a series of 12 experiments on mixed diets found as an average a heat production for 24 hours of 2238 calories and an oxygen absorption of 652.1 gm. The mean thermal quotient in this series was 3.43 Cal. per gram, which agrees very well with the theoretical value based upon a mixed diet. At 18° C. the heat value per liter of O_2 would be 4.61 Cal.

TABLE 3

THERMAL QUOTIENT OF O_2 BASED UPON EXPERIMENTS ON MAN (ATWATER AND BENEDICT)

Exp. No.	Heat Measured Cal.	Weight of O_2 Absorbed Gms.	Thermal Quotient Cal. per Gm. O_2
1	2379	708.0	3.36
2	2279	681.2	3.34
3	2085	603.2	3.45
4	2403	689.0	3.48
5	2287	664.8	3.44
6	2309	658.1	3.50
7	2151	628.5	3.42
8	2193	630.2	3.47
9	2176	659.7	3.30
10	2244	647.5	3.46
11	2272	656.0	3.46
12	2079	600.6	3.46
Mean	2238	652.1	3.43

The greatest deviation from the mean is represented by experiment No. 9 where it is only 3.9 per cent.

In the case of a man on a lacto-vegetarian diet containing 39 gm. protein, 25 gm. fat and 265 gm. carbohydrate Atwater and Benedict found that 1800 Cal. of heat were eliminated and that the absorption of oxygen

[1] The weight of a liter of oxygen at 18° C. is 1.341 gm.; that of CO_2 is 1.804 gm.

footed up 528 grams. The thermal quotient therefore was 3.41 Cal. as against a theoretical value of 3.45 calculated from the composition of the diet. The error involved in the use of a thermal quotient of 3.43 Cal. per gram for vegetarian as well as mixed diet would not be in excess of 0.5 per cent.

The values thus far discussed were obtained upon the resting subject. Would they apply equally to a subject engaged in heavy muscular work where oxygen is utilized not merely for production of heat by combustion but also for the transformation of the food's potential energy into mechanical work? Lefèvre(*g*) has calculated the thermal quotients for many of the work experiments found in Atwater's publications and has grouped them as given in the table below. The amount of work reckoned on the basis of 24 hours was from 120,000 to 190,000 kilogrammeters.

TABLE 4

THERMAL-QUOTIENTS OF O_2 DURING MUSCULAR WORK (ATWATER AND BENEDICT)

Experiment	Heat Measured Cal.	Oxygen Absorbed Gms.	Thermal Quotient Cal. per Gm. O_2
Mean of 3 exp. on fat-rich diet	3570	1053.5	3.39
Mean of 3 exp. on CH rich diet	3699	1081.6	3.42
Mean of 8 exp. on fat-rich diet	5128	1512.7	3.39
Mean of 8 exp. on CH rich diet	5142	1465.6	3.50
Mean	4385	1278.5	3.425

It appears that the mechanical equivalent of oxygen when expressed as heat is the same as the pure combustion equivalent. This is a very significant fact for it means that the liberation of energy from combustible substances is a constant function of the oxygen absorbed whether that energy take the form at once of free heat or pass first through the form of mechanical work.

It is clear that if the oxygen absorption of a subject is known the amount of energy liberated in the body (not necessarily the amount of heat) can be found with a high degree of accuracy by simply multiplying the number of grams of oxygen by 3.43 Cal. or the number of liters at 0° and 760 by 4.90 Cal. or the number at 18° C. by 4.60 Cal.

b. *Thermal Quotient of Carbon Dioxid.*—Results not nearly so constant are obtained when the carbon dioxid elimination is employed as the basis of computing the heat production. For example, when tristearin is completely oxidized the thermal quotient of CO_2 is 3.35 Cal. per gram. When glucose is completely oxidized it is only 2.59 Cal. per gram (Table 2). Besides, it is possible to have CO_2 produced in large excess when glucose is transformed into fat, and when the heat production is very low. Under these circumstances the thermal quotient of CO_2 is given by Lefèvre at 0.3 Cal. per gram. Finally, if fat is ever converted to glucose in the body

(and the possibility of this reaction has never been disproved) the production of carbon dioxid in proportion to the amount of heat disengaged would be very small and the thermal quotient would be correspondingly high. Lefèvre(*g*) has brought together results from Atwater and Benedict's work to show that the weight of CO_2 produced for each 100 Cal. of heat eliminated from the human body is very variable. The results are given in the table below.

TABLE 5

VARIATION IN HEAT EQUIVALENT OF CO_2 (ATWATER AND BENEDICT)

Condition	Heat Measured per 24 Hrs., Cal.	Weight of CO_2 Eliminated in 24 Hrs., Gm.	CO_2 in Gm. per 100 Cal. of Heat
Inanition	2346	698.0	29.8
Resting	2287	823.5	36.0
Resting	2272	846.7	37.2
Moderate work	3420	1158.0	33.9
Severe work	5205	1657.0	31.8
Severe work	5178	1884.0	36.4

Even in experiments of long duration it is evident that the calculation of heat production upon the basis of the carbon dioxid contains an inherent error of as much as 25 per cent. In experiments of short duration the error would be even greater. In fact, of the series of experiments from which the figures given above were obtained many were performed in two hour periods so that it is possible to follow the heat as measured and the CO_2 from period to period. In spite of a perfectly uniform heat elimination the CO_2 elimination varies at times as much as 40 per cent.

c. *The Respiratory Quotient and Its Significance.*—Even though the value of the oxygen absorption in terms of heat may be fairly constant, so that for long periods the calculation of the energy production may proceed upon this basis with involvement of very slight error, the requirements of short experiments are more rigorous. For it is quite possible that an observation of, say, only 15 minutes duration made perchance soon after a meal would coincide with maximum absorption of carbohydrate; while another made some hours later might very well coincide with the maximum absorption and combustion of fat. Two such periods could not be concordant if the average thermal quotient for oxygen were used. The respiratory quotient, however, enables us to know what kind of food is being oxidized at any given time, or at least what possible combinations of combustion there may be.

If a sample of pure food, e. g., cane sugar, be placed in a bomb with oxygen and ignited, it is possible to learn the amount of combustion by analyzing the gases before and after firing. In the case of pure carbohydrate it would be found that just as much oxygen by weight has disappeared as is contained in the carbon dioxid formed. Or, since equal volumes of all gases contain the same number of molecules at the same pres-

sure and temperature, it would be found upon reduction to standard conditions that the volume of CO_2 produced had just replaced the volume of O_2 consumed.

The same method may be employed, in fact has been repeatedly employed, especially by the French students of respiratory metabolism, to examine the quality of combustion in the human body. For example, Weiss sealed a child up in a closed box containing pure air, and at the end of an hour drew off samples for analysis. The box had a capacity of 60 liters and in this amount of atmospheric air the child could subsist for several hours. Comparing then the composition of the air at the end of an hour with the composition at the beginning it was found that, in certain instances, the carbon dioxid produced had exactly replaced the oxygen utilized by the child. The observer correctly inferred that carbohydrate had been the source of the energy liberated by the combustion; for in carbohydrate there is nothing to unite with oxygen except carbon, the hydrogen present being already cared for by the intramolecular oxygen. In this instance the relation by volume of the carbon dioxid produced to oxygen absorbed would be 1.0. This relationship in metabolism is the *respiratory quotient.*

The actual chemical reactions involved in the combustion of the several organic foodstuffs will now be given and the respiratory quotients typical of each deduced therefrom.

Glucose, the normal sugar of the blood is oxidized thus:

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O$$

The relation of CO_2 by volume to the O_2 is $\frac{6\ CO_2}{6\ O_2} = 1.0$, or the relation by weight of the O_2 in CO_2 to O_2 absorbed is $\frac{6\ O_2}{6\ O_2} = 1.0$.

The respiratory quotient is unity. When a simple fat like palmitine, $C_3H_5(C_{16}H_{31}O_2)_3$ is burned, conditions are as follows: The fat may be written thus: $C_{51}H_{98}O_6$ and its combustion would proceed according to the equation:

$$C_{51}H_{98}O_6 + 72.5\ O_2 = 51\ CO_2 + 49\ H_2O$$

The relation of CO_2 by volume to the O_2 is $\frac{51}{72.5} = 0.703$, which is the respiratory quotient. With a simpler fat such as the butyrate: $C_3H_5(C_4H_7O_2)_3$, the relationship would be quite different, owing to the relatively larger quantity of O_2 already present in the molecule. Thus: $C_{15}H_{26}O_2 + 18.5\ O_2 = 15\ CO_2 + 13\ H_2O$. The respiratory quotient would be $\frac{15}{18.5} = 0.81$. Food fats are for the most part composed of the glycerides of palmitic, stearic, and oleic acids, an average composition on the percentage basis being 76.5 per cent C; 11.9 per cent H; and 11.6

per cent O. One hundred grams of such fat would require 288.6 gm. O_2 in addition to that already present in the molecule for complete conversion to CO_2 and H_2O. There would be produced 280.5 gm. CO_2. The relationship of $\frac{CO_2}{O_2}$ is $\frac{280.5}{288.6}$ and this divided by $\frac{44}{32}$, the molecular weight, or multiplied by $\frac{8}{11}$ would give the respiratory quotient $= 0.706$. A slightly simpler calculation, as noted above, is to determine the weight of O_2 necessary to form CO_2 (in this case 204.0 grams) and divide this directly by the weight of total O_2 required; thus: $\frac{204.0}{288.6} = 0.706$.

The respiratory quotient of all food fats is in the neighborhood of 0.71. The same is true also of body fat. Hence whether pure body fat or pure food fat were being burned, the R. Q. would be approximately 0.71. As a matter of fact, this quotient is probably never actually produced under normal conditions; for there is always some protein being destroyed, and, since under the conditions of high fat combustion, whether from starvation or excessive fat ingestion this small amount of protein is readily oxidized, there is a mixed quotient contributed in small part by the oxidation of protein and in large part by the oxidation of fat. On the assumption that the protein quotum of energy production is 15 per cent and the other 85 per cent is from fat, Magnus-Levy estimates that the actual respiratory quotient should be 0.722, while if the remaining 85 per cent is produced from carbohydrate, the quotient should be 0.971.

The respiratory quotient of proteins will, of course, depend upon the exact formula employed; but since all proteins are made up of amino acids, the exact relationship can best be appreciated by starting with a single amino acid. If alanin is given to an animal, it will be oxidized after deamination, as follows:

$$CH_3.CHNH_2.COOH + HOH = CH_3.CHOH.COOH + NH_3$$
$$CH_3.CHOH.COOH + 3\,O_2 = 3\,CO_2 + 3\,H_2O$$

The respiratory quotient of this reaction would be 1.0 since the volume of O_2 is just equal to the volume of CO_2 produced. But the NH_3 is not yet disposed of. It cannot remain in the body as NH_3 and it cannot be eliminated as a gas, for the lungs are not permeable to NH_3 even if it could be carried in the blood as gas. Actually, the NH_3 will unite with the CO_2 to form ammonium carbonate, thus:

$$2\,NH_3 + CO_2 + H_2O = (NH_4)_2\,CO_3$$

Later, this is converted to urea, thus:

$$\begin{matrix} NH_4\diagdown \\ \qquad CO_3 \\ NH_4\diagup \end{matrix} - 2\,H_2O = \begin{matrix} NH_2\diagdown \\ \qquad CO \\ NH_2\diagup \end{matrix}$$

The net result would be that for each two molecules of alanin, yielding 2 molecules of NH_3, one molecule of CO_2 would fail to appear in the expired air, but would be eliminated as urea or water. Hence, for 6 molecules of O_2 absorbed, only 5 would come back as CO_2 and the true R. Q. of alanin would be $5/6 = 0.833$. If all proteins were made up of amino acids as simply as this, the R. Q. for their combustion would be as easily computed. The respiratory quotient of glycocoll would be 0.75; that of leucin would be 0.73. But that of lysin containing two NH_2 groups and requiring, therefore, one molecule of CO_2 for elimination of the N as urea for each single molecule of the substance, would be only 0.71. The more diamino acids contained in a protein, therefore, and the more leucin, the lower would be the respiratory quotient. With gelatin, which contains a high percentage of glycocoll, one might expect a somewhat higher quotient than with casein which contains no glycocoll and a much larger amount of leucin. Taking an example of a highly synthetized protein such as l-leucyl-triglycyl-l-leucyl-triglycyl-l-leucyl-octoglycyl-glycin, which was put together by E. Fischer and whose exact chemical structure is therefore known, we find that 45 molecules of O_2 would be necessary to produce complete combustion; that 9 molecules of CO_2 would be needed to remove the NH_2 in the form of $(NH_4)_2CO_3$; and that when this ammonium carbonate breaks down by dehydration to form urea, none of the carbon would return to the respiration and none of the oxygen would be available for combustion. The R. Q. therefore would be 0.81.

Taking the elementary analysis of protein of the human body and adopting the percentages used by Magnus-Levy we get the following composition after making allowances for the elements which would appear in the urine and the feces: C, 38.6 per cent; H, 4.24 per cent; O, 9.24 per cent. For the combustion of 100 grams of such protein, 127.6 gm. O_2 in addition to that already present in the molecule would be needed and 141.5 gm. CO_2 would be formed. Taking the ratio of the oxygen in CO_2 (102.9 gm.) to the total oxygen required, the quotient is 0.807 or by the longer calculation $\frac{141.5 \text{ gm. } CO_2}{127.6 \text{ gm. } CO_2} \times \frac{8}{11} = 0.807$. The respiratory quotient of a complete protein such as is ordinarily used in rebuilding the human tissues, but which, because it is not needed for this purpose, is oxidized as completely as it is possible to oxidize protein in the body, is thus approximately the same as that for alanin. We may think of this amino acid as representing the type of fuel available when protein is burned.

Laulanié(*c*) in 1898 gave a very simple method of calculating the thermal quotient for oxygen from the respiratory quotient. This method is strictly applicable however only under conditions where the metabolism of protein is entirely negligible, or is calculated independently and suitable

deduction made from the total oxygen absorbed. The method follows: Let a be any R. Q. less than 1.0. Then Vol. $CO_2 = a$ Vol. O_2. Let x be the part of O_2 used in combustion of carbohydrate, and Vol. $O_2 - x$ the part utilized in combustion of fat. Then Vol. $CO_2 - x$ is the CO_2 resulting from combustion of fat. The R. Q. of fat being 0.7 it follows that $\frac{\text{Vol. } CO_2 - x}{\text{Vol. } O_2 - x} = 0.7$ or, $a \frac{\text{Vol. } O_2 - x}{\text{Vol. } O_2 - x} = 0.7$. From which $x = \frac{(a - 0.7)\,\text{Vol. } O_2}{0.3}$ which is the quantity of O_2 utilized in combustion of carbohydrate. The remainder, Vol. $O_2 - x = \frac{(1 - a)\ \text{Vol. } O_2}{0.3}$ is the part used in combustion of fat. Calling this value y we have: for carbohydrate $x = \frac{a - 0.7}{0.3}$ and for fat $y = \frac{1 - a}{0.3}$. For example where a is 0.9 $x = \frac{2}{3}$ and $y = \frac{1}{3}$. The thermal quotient of oxygen at 0° and 760 (page 556) would then be $5.09 \times \frac{2}{3} + 4.7 = 4.96$ Cal. per liter, or, 4.65 Cal. per liter at 18° C.

A single example of the use of the respiratory quotient for calculation of the heat production by means of the thermal quotient for oxygen will be given. Lefèvre(*f*) separated the inspired air from the expired air of a subject in complete muscular repose by means of a pair of Müller valves (page 533). The expired air was measured and subsequently analyzed. In a one-hour period the amount of oxygen absorbed measured at 18° C. was 13.73 liters. The R. Q. was 0.89, which the author states corresponds to a combustion in which out of three molecules of oxygen absorbed, two served for oxidation of carbohydrate and one for oxidation of fat. The mean thermal quotient then would be $4.77 \times 2 + 4.41 = 4.65$ Cal. per liter. The heat production was ($13.73 \times 4.65 =$) 63.8 Cal. per hour or about 1500 Cal. in 24 hours. This minimal metabolism was confirmed by Lefèvre by direct calorimetry. It corresponds well with later determinations of the *basal metabolism* (see page 607).

4. Calculation of Heat Production from the Respiratory Exchange and the Urinary Nitrogen.—The method outlined above even when the respiratory quotient is known is defective in that it does not take account of the protein metabolism which is always taking place. Apparently the first to attempt an improvement of the method by making allowance for the protein metabolism was Kauffmann. His paper was followed three months later by one from Laulanié who had developed similar improvements quite independently.

a. *The Method of Successive Thermal Quotients.*—Instead of relying upon a mean thermal quotient for oxygen which answers very well for

long experiments Kauffmann undertook to find an exact heat equivalent for any particular short period by what he called *successive* thermal quotients. This means only that he partitioned the oxygen to the several organic foodstuffs and multiplied by their respective thermal quotients. For example in an experiment on a dog subjected to a prolonged fast he found that the animal had absorbed in 1 hour 5.992 liters of O_2, had given off 4.494 l. of CO_2 and eliminated 0.1983 gm. N in the urine. The R. Q. was 0.75. The nitrogen corresponded to ($0.1983 \times 6.25 =$) 1.239 gm. protein burned, which in turn required 1.72 gm. of O_2 to oxidize it to the stage of urea (page 555). Subtracting this from the total oxygen (5.992 l. = 8.57 gm.) there remained 6.85 gm. for combustion of fat. The heat production was found as follows:

1.72	gm. O_2	$\times 3.19 =$	5.486 Cal.
6.85	" "	$\times 3.29 =$	22.536 "
		Total	28.022 "

Applied to the human subject in good nutritive condition and subsisting on a mixed diet the method would be a little more complicated. Thus Arthus reports the metabolism of a man for 24 hours:

O_2 absorbed = 496 l. or 709 gm.
CO_2 eliminated = 463 l. or 912 gm.
N in urine 17.35 gm. = 108.44 gm. protein

The protein would require the absorption of 151 gm. O_2 and elimination of 180 gm. CO_2.

$709 - 151 = 558$ gm. O_2 or 390 l.
$912 - 180 = 732$ " CO_2 or 371 l.

The remainder represents the metabolism of carbohydrate and fat.

Let x be the volume of O_2 for combustion of fat and y the volume of CO_2 resulting. Let z represent the volume of O_2 and CO_2 for combustion of carbohydrate.

Then $y\,x = 0.70$
$x + z = 390$ l.
$y + z = 371$ l.
From which $x = 63.33$ liters O_2
$y = 44.33$ " CO_2
$z = 326.33$ " O_2 and CO_2

The weights of a liter of O_2 at 760 mm. Hg and 0° being 1.43 grams, the apportionment of O_2 would be as follows:

For fat (63.33 x 1.43 =)	90.56 gm.
" carbohydrate	467.12 "
" protein	151.0 "

The heat production then would be:

90.56 × 3.29[4]	= 297.9	Cal.
467.12 × 3.56	= 1662.9	"
157.0 × 3.19	= 481.7	"
Total	2442.5	"

Kauffmann confirmed the correctness of this method of calculation by means of a calorimeter (p. 571) suitable for dogs. His results may be summarized thus:

TABLE 6

Exp. No.	Heat as Calculated	Heat as Measured
I	27.4 Cal.	27.9 Cal.
II	30.8 "	30.0 "
III	43.7 "	44.0 "
IV	39.1 "	38.1 "
V	37.7 "	37.4 "
VI	40.2 "	39.0 "
Mean	36.07 "	36.07 "

The discrepancy between the two methods is only one per cent.

b. *Method of Zuntz and Schumberg* (*b*).—In their study of the metabolism of a marching soldier Zuntz and Schumberg developed a somewhat different method of calculation based, however, upon essentially the same principles as the method of Kauffmann. All calculations are on the basis of one hour.

The N in the Urine (per hour) (a) × 2.56 = C from protein in the respiration.

The CO_2 output in grams per hour × 3/11 = C output in grams per hour.

The C of respiration — C of protein in respiration = C of carbohydrate and fat in respiration (b).

N in urine × 8.45 = O_2 from protein in respiration.

Total O_2 absorbed — O_2 from protein = O_2 absorbed for carbohydrate and fat (c).

The O_2 for oxidation of one gram of fat = 3.751[4] (average).

The O_2 for oxidation of one gram of CH = 2.651 (average).

Let x = number of grams C from fat (1 gm. C from fat = 12.3 Cal.).

Let y = number of grams C from CH (1 gm. C. from CH = 9.5 Cal.).

$x + y = b$. (1 gm. N. from Prot. = 26.0 Cal.)

$3.751\ x + 2.651\ y = c$

Solving for x and y,

a × 26	= Cal. from Prot.
x × 12.3	= Cal. from fat.
y × 9.5	= Cal. from CH
Total	= Cal. per hour.

[4] Compare the thermal quotients (see page 556).

5. The Non-Protein Respiratory Quotient.—It was but a step from the method just given to a simpler calculation based upon a table giving the heat values of oxygen or carbon dioxid for the non-nitrogenous combustion.

The respratory exchange due to protein is thus given by Lusk (*h*).

TABLE 7

100 gm. meat contain	52.38 gm. C.	7.27 gm. H.	22.68 gm. O.	16.65 gm. N.	1.02 gm. S.
Eliminated in the Urine	9.406 " "	2.663 " "	14.099 " "	16.28 " "	1.02 " "
In the Feces.....	1.471 " "	0.212 " "	0.889 " "	0.37 " "	
Leaving for respiratory metabolism...	41.50 " "	4.40 " "	7.69 " "		
Deducting intramolecular water	.....	0.961 " "	7.69 " "		
	41.50 gm. C.	3.439 gm. H.			

To oxidize these amounts of carbon and hydrogen would require 138.18 gm. O_2 and there would be produced 152.17 gm. CO_2. From which it may be deduced that for every gram of nitrogen appearing in the urine from meat there would be absorbed from the breath (138.18 ÷ 16.28 =) 8.45 grams of oxygen, and there would be produced (152.17 ÷ 16.28 =) 9.35 grams of carbon dioxid. Hence by multiplying the nitrogen elimination in the urine whether of an hour or a day by these factors and subtracting from the total oxygen absorbed and carbon dioxid eliminated the *non-protein respiratory quotient* is obtained.

By a method entirely analogous to that of Laulanié (page 562) it is possible to learn the heat values of oxygen for each value of this respiratory quotient. Zuntz and Schumberg (*b*) prepared a table setting forth these values which is now widely employed. As reproduced here the heat values of both oxygen and CO_2 per liter of the gas at 0° and 760 mm. Hg may be read off for any value of the non-protein R. Q. given to two places.

It will be noted that the values for pure fat (R. Q. 0.71) and pure carbohydrate (R. Q. 1.0) combustion differ but slightly from those of Lefèvre given in Table 2 (page 556).

The calculation of the heat production from the respiratory exchange and the nitrogen in the urine involves then the following steps:

(1) Determination of total O_2 and CO_2 of respiration in grams.
(2) " " " N in the urine.
(3) Multiply N of urine by 8.45 = O_2 for protein.
(4) " N " " " 9.35 = CO_2 " "
(5) Subtract these values from total O_2 and CO_2.
(6) Convert to volume and get Non-prot. R. Q.

TABLE 8

HEAT VALUE OF OXYGEN AND CARBON DIOXID FOR DIFFERENT NON-PROTEIN RESPIRATORY QUOTIENTS

Caloric value of 1 liter at 0° and 760 mm.			Caloric value of 1 liter at 0° and 760 mm.		
R. Q.	CO_2	O_2	R. Q.	CO_2	O_2
0.70	6.694	4.686	0.86	5.669	4.875
0.71	6.606	4.690	0.87	5.617	4.887
0.72	6.531	4.702	0.88	5.568	4.900
0.73	6.458	4.714	0.89	5.519	4.912
0.74	6.388	4.727	0.90	5.471	4.924
0.75	6.319	4.739	0.91	5.424	4.936
0.76	6.253	4.752	0.92	5.387	4.948
0.77	6.187	4.764	0.93	5.333	4.960
0.78	6.123	4.776	0.94	5.290	4.973
0.79	6.052	4.789	0.95	5.247	4.985
0.80	6.001	4.801	0.96	5.205	4.997
0.81	5.942	4.813	0.97	5.165	5.010
0.82	5.884	4.825	0.98	5.124	5.022
0.83	5.029	4.838	0.99	5.085	5.043
0.84	5.774	4.850	1.00	5.047	5.047
0.85	5.721	4.863			

(7) Read off heat value of Non-Prot. R. Q. from table.
(8) Multiply by liters of Non-Prot. O_2.
(9) Multiply N of Urine by its heat value (26.51 Cal. for meat diet).
(10) Add 8 and 9 for total heat production.

B. Direct Calorimetry

Without the disintegration of organic substances accompanied by a diminution of potential energy life is impossible. One of the forms which the liberated energy inevitably takes is heat, and in the resting organism, i. e., not transferring energy in the form of mechanical work to other objects, all of the energy finally takes this form. The quantity of heat, therefore, becomes a measure of vitality.

We have seen that this measure can be applied in an indirect way by measuring the potential energy of the foodstuffs or by assigning a heat equivalent to a unit of oxygen absorbed. But this method is based upon certain assumptions which are always open to debate, namely, the assumption that specific chemical changes are always accompanied by the same transformations of energy and the assumption that the law of the conservation of energy applies to all chemical transformations in the animal body. Most authorities are agreed that for these reasons the direct measurement of heat generated in the living organism is at least more authoritative even though the accomplishment of this end may be beset with great difficulties. Krogh(*c*) states that "With the recent advances in calorimetric methods due to Atwater and Benedict, Rubner and especially A. V. Hill,

there is every reason to think that direct determinations of the total metabolism will be preferred to the indirect in many cases, and all classes of animals, as it is undoubtedly preferable theoretically." Lefèvre(g) says, "Aussi bien la calorimétrie physique est á la base de toute recherche de calorimétrie biologique." And Rubner(p) points out that "Die urprüngliche Auffassung des Tierlebens als eine Verbrennung unter oxydativen Abbau der Stoffe hat der allgemeine energetischen weichen müssen, denn die letztere umfasst auch jene primativen Lebensformeln bei Bakterien und Hefe wo Spaltungsvorgänge ohne Beteiligung des Sauerstoffs die Quelle der Energie für die lebende Substanz bilden." Rubner also draws attention to the fact that in all organisms there are fermentative reactions not directly related to the needs of the living substance, which nevertheless lead to the development of heat. Such heat would represent pure loss of energy unless, as in the higher animals which possess a specific chemical regulation, it were turned to account in the maintenance of the body temperature. The different fermentative processes therefore come within the field of calorimetric investigation. The production of living substance in the growing organism on the other hand is of the nature of fermentative changes which themselves involve no storage or liberation of energy, and yet they are dependent upon energy changes and indeed may to a degree be measured by the intensity of the oxidative capacity of the organism.

Calorimetry as related to living organisms has two distinct fields: (1) the physical measurement of the energy stored in the animal tissues and in all chemical compounds which may serve the animal as food, likewise the energy residual in the excretory substances rejected by the cells; (2) the measurement of the energy set free as heat during the life processes.

I. The Heat of Combustion

The unit of heat which has been employed for nearly a century is the Calorie of Regnault, i. e., the amount of heat necessary to raise 1 kilogram of water from 0° to 1° C. This is the kilo-calorie written with a capital C. The small calorie written "cal," called also the gram-calorie, is the amount of heat necessary to raise 1 gram of water from 0° to 1° C. The calorie more commonly used to-day is somewhat smaller than this, namely, the amount necessary to raise a kilogram of water from 15 to 16° C or from 19° to 20° C. In terms of the original Regnault calorie the value of the calorie at higher temperatures is given by Longuinine as follows:

$$18° = 0.9995$$
$$20° = 0.99925$$
$$22° = 0.99915$$
$$25° = 0.99930$$

Berthelot (*a*) introduced the method of burning substances in oxygen at high pressure, but because of the high cost of the apparatus it did not come into general use for some years after it was described. The essential parts of the original apparatus were a double-walled copper vessel filled with water in which was immersed the vessel capable of holding the oxy-

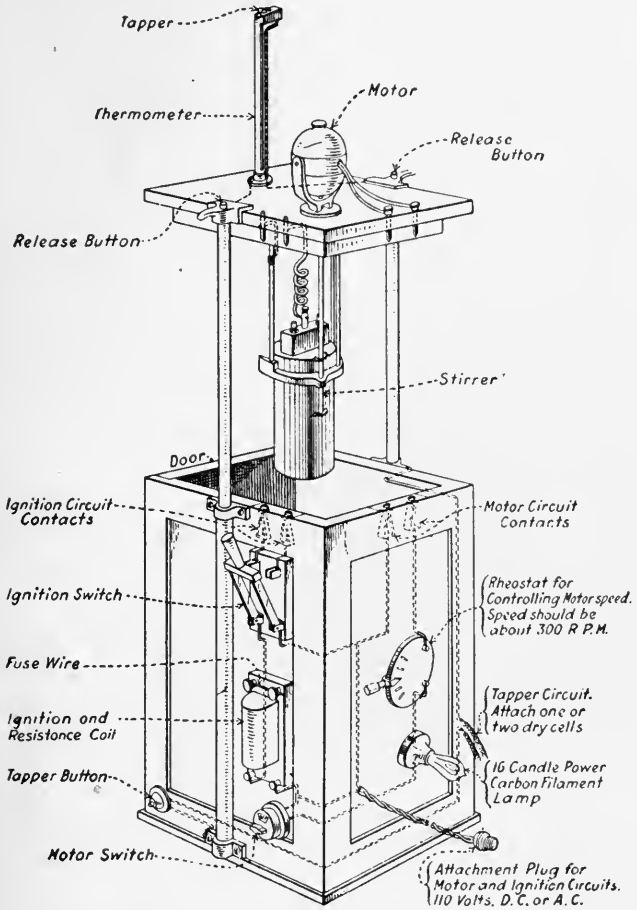


Fig. 23. The bomb calorimeter of Riche for use with Berthelot bomb. The Weinholdt cup which is placed inside the box and into which the pump is lowered is not shown.

gen under high pressure together with the substance to be burned. This vessel constructed of heavy steel nickeled on the outside and lined with platinum became known as the Berthelot bomb, and whatever the modification from the original pattern it is still known by the inventor's name. The outer container filled with water is the calorimeter proper. A successful modern construction is that of Riche shown in Fig. 23. It consists of a

wooden box lined with a heavy layer of compressed cork board. Inside this is a Weinholdt vacuum cup which serves as the receptacle for water. The bomb is lowered into the water by a carriage attached to the top of the box which slides upon two metal supports at the sides. The top also carries a motor for operating a stirrer in the water and a Beckman thermometer. The substance to be burned is placed in a nickel vessel supported upon platinum wires inside the bomb. The bomb is then charged with oxygen and immersed in the water. When the temperature of the water has become constant (at about 20° C.) the combustion is started by throwing a switch which connects the house circuit with a platinum or nichrome wire inside. A standard amount of current is secured by means of a fuse wire, which burns off with just enough current to "fire" the combustible material. The reading at ignition is taken as the initial reading. This subtracted from the final reading gives the total rise. The increase in temperature multiplied by the weight of water contained in the vacuum cup (plus the hydrothermal equivalent of the apparatus) gives the total heat liberated. Certain corrections have to be applied for the heat caused by the current in firing, and for any nitric acid formed from oxidation of nitrogen. For example in burning a sample of standard cane sugar the weight of substance taken was 1.1466 grams. Weight of water in the cup was 2530 gm.

Hydrothermal equivalent	470 gm.
Water equivalent of apparatus	3000 gm.
Rise in temp. was 1.530°C.	Ignition heat — 60 cal.
1.530° × 3000 gm. = 4590 cal.	Nitric acid — 4.6 cal.
4590 — 64.6 = 4525 cal.	64.6
4525 ÷ 1.1466 gm. = 3947 cal. per gm.	

The table on page 571 compiled from various sources gives the heat value of the most important organic substances concerned in metabolism of the higher animals.

II. Animal Calorimetry

1. Forms of Calorimeters.—The various types of apparatus devised for measuring the heat eliminated by an animal body are classified by Lefèvre(*q*) into four groups: (1) those which make use of latent heats; for example, the ice calorimeter of Lavoisier and the distillation calorimeter of D'Arsonval; (2) those which depend upon the warming of a fixed quantity of water such as the calorimeters of Dulong and Laulanié for animals and the bath calorimeter of Lefèvre for man; (3) those which employ circulating mediums (air or water) to carry away the heat just as rapidly as it is produced (compensation method); such as the respiration calorim-

TABLE 9

HEAT VALUE OF ONE GRAM OF EACH SUBSTANCE IN LARGE CALORIES

Substance	Stohmann	Berthelot	Rubner	Benedict
Glycerin	4.316	.....	.....	4.323
Glucose	3.743	3.762	.....	3.739
Levulose	3.755	.....	.....	3.729
Galactose	3.722	.....	.....	.....
Cane sugar	3.955	3.962	4.001	.....
Milk sugar	3.737	3.777	.....	3.737
Maltose	3.722	.....	.....	3.776
Dextrin	.....	4.119	.....	.....
Starch	4.183	4.228	.....	.....
Palmitic acid	.....	9.265-9.369	9.745	9.318
Stearic acid	.....	9.429-9.549	9.745	9.499
Oleic acid	.....	9.511	9.334	9.423
Animal fat	9.500	.....	.....	.....
Butter	9.231	.....	.....	.....
Vegetable oil	.....	9.520	.....	.....
White of egg	5.735	5.687	.....	.....
Yolk of egg	5.841	.....	.....	.....
Beef (ext. free of fat)	5.721	5.728	5.778	.....
Veal	5.663	.....	.....	.....
Casein	5.850	5.626	.....	.....
Peptone from fibrin	5.942	.....	.....	.....
Glycogen	.....	.....	.....	4.227
Alanin	.....	.....	.....	4.401
Asparagin	.....	.....	.....	3.065
Aspartic acid	.....	.....	.....	2.882
Creatin	.....	.....	.....	4.240
Creatinin	.....	.....	.....	4.988
Cystin	.....	.....	.....	4.137
Glutamic acid	.....	.....	.....	3.662
Glycocoll	.....	.....	.....	3.110
Tyrosin	.....	.....	.....	5.915
Alcohol	.....	.....	.....	7.104
Lactic acid	.....	.....	.....	3.615
Urea	2.537	.....	.....	.....
Uric acid	2.741	.....	.....	.....

eters of Atwater and Rosa, Pompilian, and Lefèvre; and (4) those which do not absorb the heat from the subject but which record only the effects of heat in one way or another. Examples are the anemo-calorimeter or the thermo-electric calorimeter of D'Arsonval, the siphon calorimeter of Richet, and the second calorimeter of Rubner.

It is not necessary to describe more than two or three calorimeters. The first method described above has never been used in studying the metabolism of man and is now wholly obsolete. The second as a means of following the heat production of animals has fallen more or less into disfavor on account of the cooling correction which is necessary. Laulanié(*b*) has overcome this to some extent by using a pair of calorimeters of the Dulong type, running one of them, constructed in exactly the same manner as the other, as a control of the effects of environment. With this apparatus Laulanié confirmed the thermal quotients of oxygen (page 557) in an apparently satisfactory manner.

As a means of studying the heat production of man the second method has been employed in the form of a bath in which the subject could be directly immersed. The first to use this method at all successfully was Liebermeister (*a*), but his technique was subjected to very severe criticism a few years later and the method fell into disfavor until rescued by Lefèvre(*a*) in 1894. The chief objections to Liebermeister's method were: (1) that he used too large a volume of water, (2) that he read its temperature on only a single thermometer and (3) did not guard against stratifica-

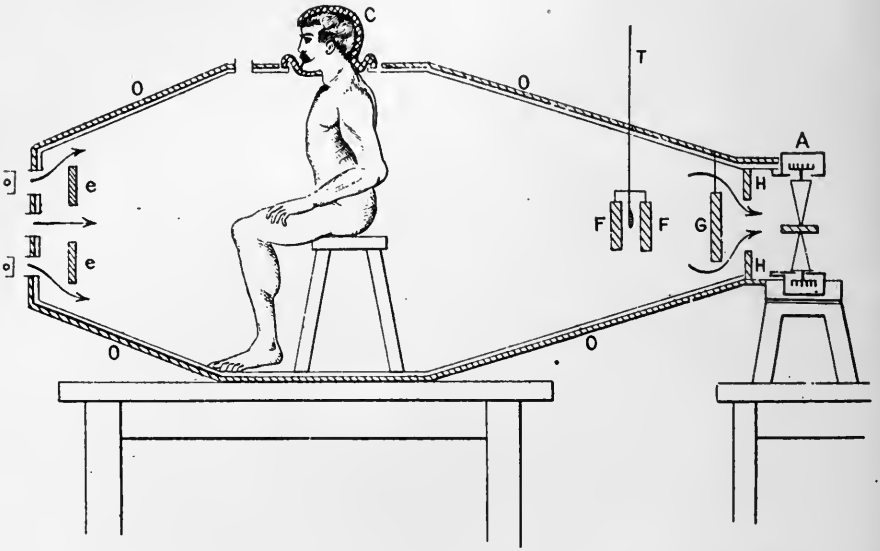


Fig. 24. The air calorimeter of Lefèvre. *OOO;* wall of the chamber; *T*, thermometer for measuring the temperature of the atmosphere after it has passed over the subject; *e*, *e*, baffle plates for distributing the air as it enters; F, G, H, baffle plates to prevent channeling of the air as it leaves the chamber; *A*, the aspirator; *C*, covering for the head which prevents radiation of heat to the exterior.

tion of the water. Lefèvre overcame these objections and proved that the heat production of a man could be measured with a high degree of accuracy by this very simple method. Even the heat of vaporization of water which ordinarily is lost through the lungs can be compensated by having the bath at 35°C. in which case the subject respires an atmosphere already saturated with moisture.

One of the simplest types of compensation calorimeters is that of Lefèvre(*e*) designed for measurement of the heat production of a man by carrying away the heat of his body just as rapidly as produced with a current of air. The calorimeter consists of a zinc chamber 3 meters long, narrow at the two ends, but broader in the middle where the subject sits on a stool (Fig. 24). Air is drawn through the chamber by means of an aspirator shown at A. The volume of air is recorded by means of an anemometer.

The increase in temperature is observed by continuous readings of thermometers placed in the inlet and other thermometers placed in the current after it has passed over the man's body. The heat elimination is found by multiplying the volume of air by factors converting it to weight, by its specific heat and by the average rise in temperature.

The two methods of Lefèvre just described are well suited for a study of the influence of environing temperature upon heat production. One has only to vary the temperature of the bath or current of air before it strikes the body to vary the cooling effect. Lefèvre combined the water-bath method with a method for obtaining the respiratory exchange.

2. The Atwater-Rosa-Benedict Respiration Calorimeter (Atwater and Benedict) (*d*).—The fundamental principles of this apparatus which was designed to measure accurately the heat elimination of a man, are as follows: The subject is confined in a heat-proof chamber through which a current of cold water is kept constantly passing. The amount of water, the flow of which is kept constant, is carefully weighed. The temperatures of the water entering and leaving the chamber are read at frequent intervals on sensitive thermometers to 0.01 of a degree. The walls of the chamber are held at such a temperature as to prevent the loss of any heat through them, and withdrawal of heat by the water current is so regulated by varying the temperature of the ingoing water that the heat brought away from the calorimeter is exactly equal in amount to the heat eliminated by radiation and conduction from the subject. This is accomplished by having accurate knowledge of the temperature of the air inside the apparatus and the temperature of the walls of the calorimeter. About 25 per cent of the heat produced by the human subject is eliminated at ordinary temperatures through vaporization of water from the lungs and the skin. This latent heat in the water of vaporization is determined by measuring the amount of water vaporized and passing in the ventilating current to the first sulphuric acid absorber. The gain in weight of this absorber is taken as the water of vaporization.

The respiration chamber of this calorimeter has been constructed in several different sizes. The original construction at Middletown, Conn., had a chamber with a cubic capacity of 5.03 cubic meters, or with the subject inside a residual air volume of 4500 liters. This apparatus was dismantled at the time the Nutrition Laboratory of the Carnegie Institution was established at Boston and in its place have been constructed a number of different calorimeters (Benedict and Carpenter(*a*)) designed for different purposes. The first of these known as the chair calorimeter (Fig. 25) has a cubic capacity of approximately 1400 liters. A second construction known as the bed calorimeter (Fig. 36) has a cubic capacity of 1347 liters. That part of the original Atwater-Rosa calorimeter which was the property of the U. S. Government was shipped to Washington and has been reconstructed into a successful calorimeter by Langworthy and

Milner. More recently calorimeters have been constructed at the Cornell Medical College (Williams, H. B.) and at Bellevue Hospital (Riche and Soderstrom) in New York. The operation of these calorimeters has been under the scientific direction of Graham Lusk. The small calorimeter at the Medical School constructed by Williams has a cubic capacity of approximately 480 liters. This calorimeter was designed for the study of metabolism in infants and children as well as of animals (Fig. 29). The large calorimeter at the hospital known as the Sage calorimeter is designed for the study of patients in a reclining, sitting or supine position and has a cubic capacity of 1123 liters. Still larger calorimeters on the same principles have been constructed by Benedict at the Nutrition Laboratory in Boston, having a capacity large enough to accommodate a man doing active muscular work, and by Armsby at the Pennsylvania State College (Armsby and Fries) designed for measuring the heat production of the larger farm animals.

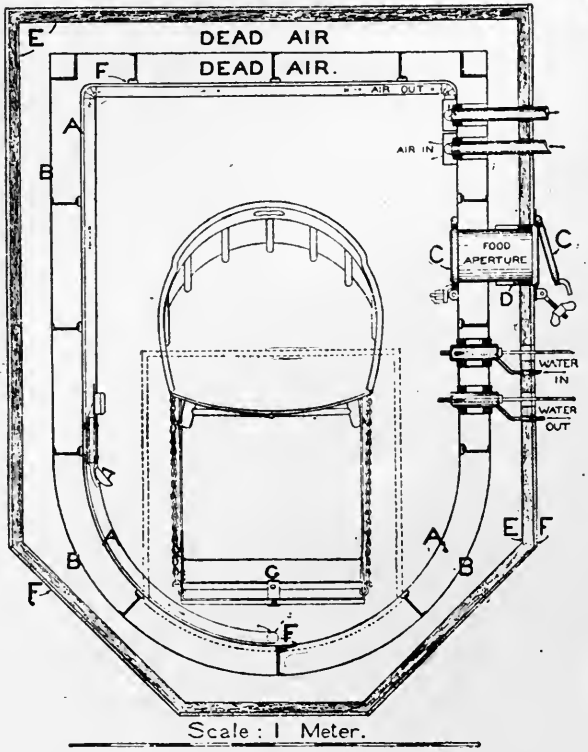


Fig. 25. Cross section of chair calorimeter of Benedict and Carpenter. *A*, copper wall; *B*, zinc wall; *C*, hair felt; *F*, asbestos lumber. At the upper right hand corner of the figure is shown the ingoing and outgoing pipes, below this at *C* the food aperture and the ingoing and outgoing water pipes with their respective thermometers. The chair is suspended from a balance carried on the frame of the apparatus above the chamber.

The wall construction is essentially the same in all of these calorimeters. The inner wall consists of copper tinned on both sides, thus permitting of soldering, while a second metal wall consists of zinc. In the cross section represented in Fig. 25, *A* represents the copper and *B* the zinc wall. Surrounding the latter and providing air insulation is a series of panels constructed of asbestos lumber lined with hair felt or with compressed cork. The whole construction, therefore, is more or less of the refrigerator type

permitting very little opportunity for radiation or conduction of heat from the inside out or from the outside in. For additional security against the radiation of heat from the calorimeters the original device of Rosa is repeated in all of these calorimeters. This is based upon the ability to hold the temperature of the zinc wall at the same level as that of the copper wall. To this end it is necessary to know first that there is a temperature difference between the zinc and copper and second to have some method

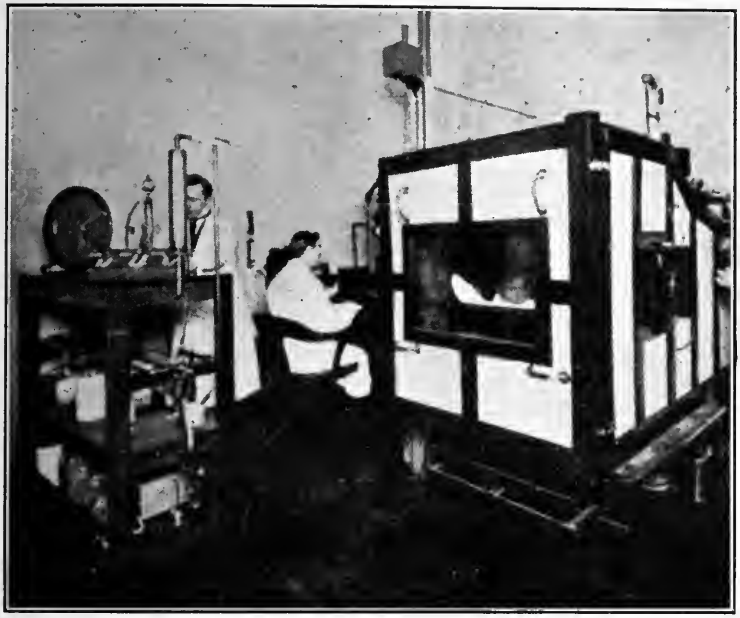


Fig. 26. The Sage calorimeter at Bellevue Hospital, New York City. The absorber table is shown at the extreme left, the observer's table in the middle and the respiration chamber at the right. Air is circulated by a blower, shown on the lower shelf of the absorber table, through overhead pipes which may be seen entering the calorimeter at the upper left hand corner. Oxygen is admitted from a cylinder shown on the extreme right.

for controlling the temperature of the former. The temperature differences of the two walls are recorded by means of electrical thermo-junctions, separate series of which are arranged in the sides, in the top and in the bottom of the apparatus (the ends of several thermal junctions can be seen in Fig. 29). A current flowing through these thermal junctions is read on a Wheatstone bridge at the observer's table and fluctuations of temperature between the two walls alters the amount of this current. To insure a cooling effect on the zinc wall a coil of copper tubes carries a thin current of water and to counteract this cooling effect a wire running in the same space and between the cooling pipes is heated by sending through it the desired amount of current. Adjustable rheostats are within reach of

the observer who reads the electrical variations on the Wheatstone bridge, so that the amount of current flowing through the several "parts" is under accurate control. Any tendency for heat to pass outward would be indicated by a deflection of the galvanometer showing that the zinc wall was cooler than the copper. Such an indication, however, would be immediately checked by turning additional current into the heating wire, thus restoring the temperature of the zinc wall to that of the copper wall and thereby preventing escape of heat.

The interior of the chamber is so arranged as to give the utmost comfort to the subject. It is obvious that if the heat were not carried away

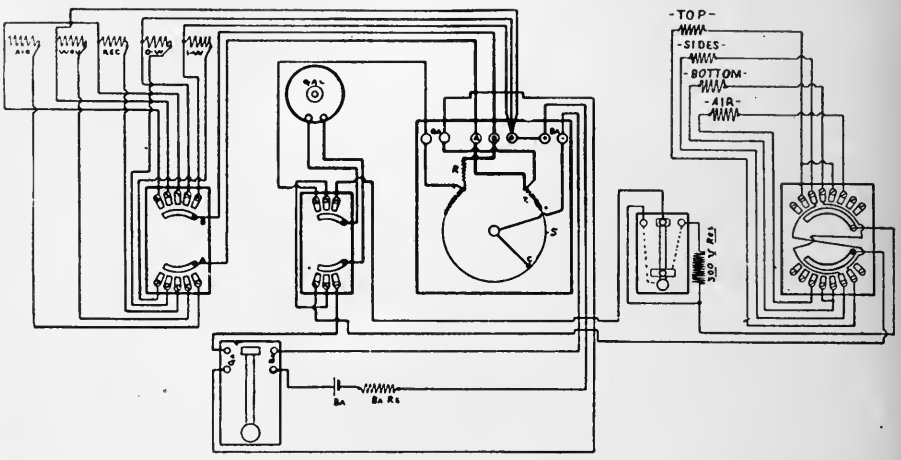


Fig. 27. The wiring diagram of the observer's table with the Sage calorimeter. In the center is the Kohlrausch bridge, to the right a tapping key with an arrangement for throwing in 300 ohms resistance when needed. This key is used in reading the thermopiles connected with the switch on the right. To the left of the bridge is a switch for connecting either thermopiles or resistance thermometers with the galvanometer. On the extreme left is the switch for the air, wall, rectal, ingoing and outgoing water thermometers, each of which contains 100 ohms.

from so confined a space the temperature would very shortly become unbearable. The heat absorbing apparatus is installed on the ceiling of the chamber. In the later constructions this absorber consists merely of a continuous grid of copper pipes covering the entire ceiling. In the Cornell and Sage calorimeters the temperature of the water as it enters is brought to the desired level by means of a Gouy temperature regulator. This device insures great constancy in the temperature of the water. With the speed of the water current properly regulated and its temperature brought to a constant level as it enters the apparatus fluctuations in the heat production will be manifested by fluctuations in the temperature of the water as it leaves the chamber. Extreme variation in the former, however, requires readjustment of both speed and temperature of entering water.

After circulating through the heat absorber the water is caught in a

meter (can) and weighed in kilograms. An electrical device under the control of an observer enables him to stop instantly the flow of water into this meter upon the termination of a period by the second hand of a clock.

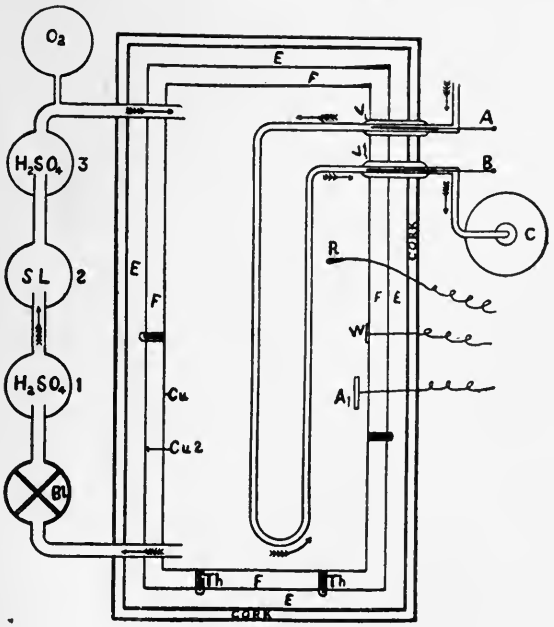


Fig. 28. Diagram of the Atwater, Rosa, Benedict respiration calorimeter as prepared by DuBois for the Sage Calorimeter.

Ventilating System:
- O_2 Oxygen introduced as consumed by subject.
- 3, H_2SO_4 to catch moisture given off by soda lime.
- 2, Soda lime to remove CO_2.
- 1, H_2SO_4 to remove moisture given off by patient.
- Bl, Blower to keep air in circulation.

Indirect Calorimetry:
- Increase in weight of H_2SO_4 (1) = water elimination of subject.
- Increase in weight of soda lime (2) + increase in weight of H_2SO_4 (3) = CO_2 elimination. Decrease in weight of oxygen tank = oxygen consumption of subject.

Heat-Absorbing System:
- A, Thermometer to record temperature of ingoing water.
- B, Thermometer to record temperature of outgoing water.
- V, Vacuum jacket.
- C, Tank for weighing water which has passed through calorimeter each hour.
- W, Thermometer for measuring temperature of wall.
- A_1, Thermometer for measuring temperature of the air.
- R, Rectal thermometer for measuring temperature of subject.

Direct Calorimetry:
- Average difference of A and B × liters of water + (gm. water eliminated × 0.586) ± (change in temperature of wall × hydrothermal equivalent of box) ± (change of temperature of body × hydrothermal equivalent of body) = total calories produced.
- Th. thermocouple; Cu, inner copper wall; Cu_2, outer copper wall; E, F, dead air-spaces.

The average rise in temperature of the numerous readings which have been taken during the period multiplied by the weight of the water gives the amount of heat eliminated by radiation and conduction and carried

away by the water current. To this must be added the latent heat in the water of vaporization and any heat stored in the body itself.

For the measurement of this latter quantity an electrical resistance thermometer is inserted into the rectum to a depth of 10 or 12 cm. Fluctuations in the body temperature can thereby be followed accurately by readings on the Wheatstone bridge. If the body temperature rises during the course of a period of observation the amount of heat stored is found by multiplying the rise in temperature by the weight of the body and by the specific heat of the animal body (0.83). Should the body temperature fall, heat will be given up to the calorimeter and may be deducted by a similar calculation.

The temperature of the ingoing air must likewise be adjusted so as to be at all times equal to the temperature of the outgoing air; otherwise, heat would be added to or taken away from the chamber by the air current. Thermal junctions are so placed as to have one terminal in the outgoing air and the other in the ingoing air immediately adjacent to the calorimeter so that any difference in temperature of the two air currents is instantly detected by connecting the circuit with the galvanometer. A cooling effect in the ingoing air is brought about by means of a continuous current of water running at a very slow rate against which a warming effect produced by an electric lamp is kept in action.

Finally heat may be stored in the calorimeter itself. To detect such a change resistance thermometers are attached to the inner walls of the calorimeter and if the temperature of these walls rises or falls between the beginning and the end of an experiment a correction is made. With the chair calorimeter it has been found that 19.5 Calories of heat are absorbed when the inner wall rises one degree of temperature. Conversely, 19.5 Calories are lost by the wall when the temperature falls one degree. This quantity is known as the hydrothermal equivalent of the calorimeter. For the bed calorimeter of Benedict the hydrothermal equivalent is 21 Calories; for the Sage calorimeter at Bellevue 19 Calories. When all of these corrections are made the result gives the amount of heat actually produced by the body in the period of observation.

a. *Control Tests.*—A calorimeter must be very carefully controlled as regards its heat measuring capacity. What is known as a "heat check" is run in the following manner: A current of electricity of known voltage is run through a resistance coil placed inside the respiration chamber. To secure uniformity in the electrical current and therefore in the amount of heat dissipated, Williams used an accumulator battery as a source of current. This battery was of sufficiently large capacity (about 45 ampere-hours) to deliver the required amount of energy over periods of four or five hours without much diminution in voltage. The current passes from the battery through a ballast resistance, then through the heat coil and back through a standard resistance. A precision milli-voltmeter measures

the fall of potential across the terminals of the standard resistance and serves to determine the current. From the heating coil in the chamber a pair of wires runs out to a voltmeter. A key is provided in this circuit so that the voltmeter may be connected momentarily to determine the fall of potential across the terminals of the heating coil. The reading of the millivoltmeter is maintained constant by manipulation of the ballast resistance

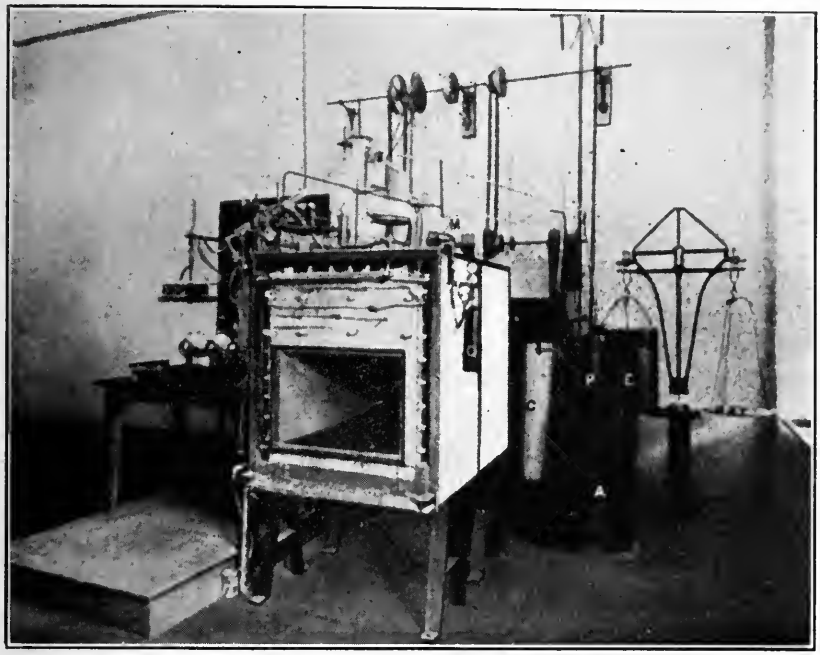


Fig. 29. The small calorimeter at Cornell University Medical College shown in process of construction. The observer's table is at the extreme left. The Gouy regulator is shown as a cubical box on top the calorimeter. The arrangement of heating and cooling elements on the outside of the zinc wall is shown at the open end of the calorimeter. The water meter *E*, suspended on a balance is shown at the extreme right. The tank supplying the heat absorber with water under constant pressure is shown at the extreme top of the picture. Water passes from this tank through a pipe to the Gouy regulator, thence to a reheater at the upper left hand corner of the calorimeter, thence through the heat absorber which is a grid of pipes on the ceiling of the inner chamber, thence back to the meter. From the waste tank, *A*, water is pumped up again into the pressure tank.

and the voltmeter is read several times during each period of the experiment. The heat dissipated is given by multiplying together the numbers expressing the fall of potential across the terminals of the heating coil (in international volts), the current in amperes and the time in seconds and dividing by the number expressing the mechanical equivalent of heat at the temperature of the flowing water. For example in a heat controlled experiment performed with the small respiration calorimeter on May 6th, 1911, Williams obtained the following results: The strength of current,

I was 2.1 amperes. The fall of potential across the terminals of the heating coil was 5.79 volts and the time for each period was 3500 seconds. The heat is given by the product E. I. t $\times$ 0.2393 = 10,470. This is expressed in small calories and is equal to 10.47 large calories. The following is a tabulation of this experiment.

TABLE 10

Hour	Calories Calculated	Calories Found	Error in Cal.
1	10.47	10.64	0.17
2	10.47	10.55	0.08
3	10.47	10.64	0.17

The advantage of this sort of a check experiment is that the measurements can be made very accurately, rapidly and in short periods. It is customary in making such checks to place the resistance coil in the calorimeter and make the connections. The current is then passed through the coil and simultaneously the water is started flowing through the heat absorbing system and the whole calorimeter is adjusted in temperature equilibrium. As soon as possible when the temperature of the air and walls is constant and the thermal junction system in equilibrium, the exact time is noted, and the water current is deflected into the water meter. At the end of the first hour, the usual length of a period, the water current is deflected from the meter, the water weighed and the average temperature difference of the water is obtained by averaging the results of all the temperature readings during the hour. Usually during an experiment of this nature records of the water temperature are made every four minutes. Occasionally, when the fluctuations are somewhat greater than usual, records are made every two minutes. Tests with the chair calorimeter of the Nutrition Laboratory made in January, 1909, show between the heat developed inside the apparatus in the electric coil and the heat as measured by the water current with corrections a discrepancy of about 0.5 per cent (Benedict and Carpenter (*a*)). A series of electric checks made upon the Sage calorimeter by the same method shows a total error for the entire series of less than 0.4 per cent (Riche and Soderstrom).

Another method of checking the heat measuring capacity of the calorimeter is known as the "alcohol check." In this method alcohol is burned inside the apparatus by means of a small alcohol lamp, the rate of flow of the alcohol being made as nearly constant as possible and the amount consumed in a period of observation being carefully recorded upon a finely graduated burette or by weighing. In planning such a test to ascertain the magnitude of the errors which are likely to occur in using the apparatus with subjects of known size it is of importance to provide that the amount of alcohol consumed per hour shall be enough to dissipate approximately

the same amount of heat as the subject would be expected to eliminate in a given time. With an experimental apparatus the error will be, assuming a uniform technique, about constant in absolute amount so that the total error will diminish as the total quantity measured increases.

When the rate of flow of the alcohol to the lamp has been adjusted so that it is fed into the burette just as rapidly as consumed therefrom by the lamp, the apparatus is sealed and after a preliminary period during which the calorimeter is brought into equilibrium, the burette is read, the supply bottle from which the alcohol is fed into the burette is changed for another which has been weighed, and the experiment starts in the usual way.

To insure complete combustion of the alcohol it is necessary to employ a lamp so constructed that the region of the edge of the wick will always be sufficiently hot to insure immediate ignition. Williams finds that by using a short piece of hard glass tubing for the top of the burner and a wick of a glass wool the difficulties attending the combustion of alcohol are most readily overcome.

The specific gravity of the alcohol must be determined with a high degree of precision after which the theoretical amounts of heat, carbon dioxid and water which the known combustion will generate may be calculated. Likewise, the amount of oxygen necessary to support this combustion. In the case of the water one must make a correction for the amount of water of dilution present in the alcohol. The heat of combustion of alcohol has been determined a great many times. As the result of 25 observations with the bomb calorimeter Atwater and Rosa found the heat of combustion of pure ethyl alcohol to be 7.067 large calories per gram. This figure is generally employed in this country. In all of the different calorimeters of Atwater, Rosa and Benedict here described the correspondence between the amounts of heat generated by the alcohol and the heat actually measured has been very close. For example, in a long series of experiments of three or four hours' duration the average error with the Sage calorimeter for the heat of combustion was 0.9 per cent, for the oxygen absorption 1.6 per cent, and for the carbon dioxid elimination 0.6 per cent.

3. The Emission Calorimeters.—The fourth group of calorimeters according to the classification of Lefèvre are those which do not absorb the heat but allow it to escape into the external medium. Because of this feature the name *calorimèters déperditeurs,* or emission calorimeters, was proposed by D'Arsonval(*a*), who devised several different types. Some of these calorimeters have single walls and the effect of the heat generated within is recorded in some way. In the so-called anemo-calorimeter of D'Arsonval the subject stands inside a tent-like cubicle which has a narrow chimney or ventilator at the top. In the chimney is a delicate wind-gauge. The heat from the man's body induces a strong convection current which is free to enter the cubicle below and which sets the wind-gauge in

rapid motion. By calibration of the apparatus with known sources of heat it is possible to determine the heating effect of the live subject.

Another group of these calorimeters have double-walls, between which is a cushion of air. The effect of heat generated within the chamber is recorded by expansion of this air cushion. Among those employing this principle of registering the effect of heat are the siphon calorimeter of Richet (*b*) (Fig. 30) and the second calorimeter designed by Rubner (*f*) (Fig. 31). Both these calorimeters have rendered extremely important service to physiological science for it was by means of the former that Richet made his contributions on the relation of heat production to body size and it was by means of the latter that Rubner first proved with a high degree of

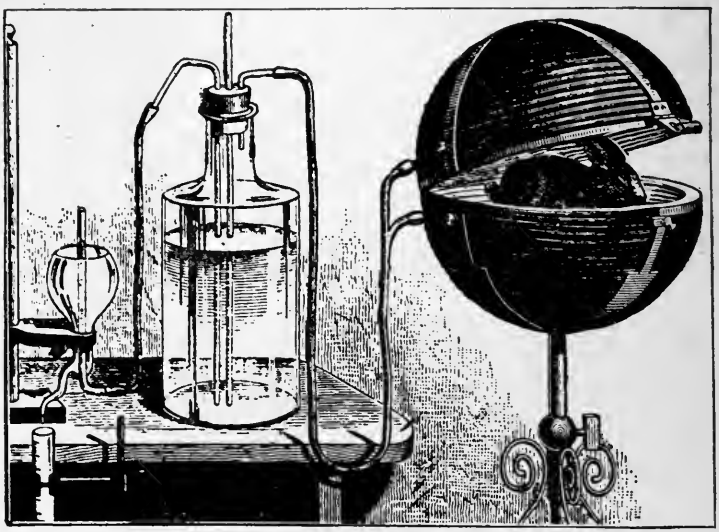


Fig. 30. Richet siphon calorimeter. For description see the text.

precision that the law of the conservation of energy applies to the animal body (see page 584). The siphon calorimeter is very simple in principle. The space between the walls of the base and cover between which the rabbit in the figure is placed communicate by a common tube with a pressure bottle containing about three liters of water. A siphon from this bottle terminates in a funnel-like vessel which catches the overflow and delivers it into a burette. By expansion of the air water is forced into the measuring limb of the siphon or over into the burette. By calibration of the apparatus with known sources of heat the heat of the animal body can be determined. It should be noted that an apparatus of this sort takes no account of the heat of vaporization.

Rubner's apparatus is a respiration calorimeter. It is ventilated in the same manner as the original Pettenkofer apparatus, and determines directly only the water and carbon-dioxid. The heat-measuring device

consists of a constant temperature bath of water in which the respiration chamber is immersed. A cushion of air immediately surrounds the chamber whose walls are of metal. The heat of the animal's body (dog) passes readily through the metal and causes the air to expand. The expansion is recorded by means of a spirometer which registers its movements graphically on a white surface (in Fig. 31 two spirometers may be seen on a shelf

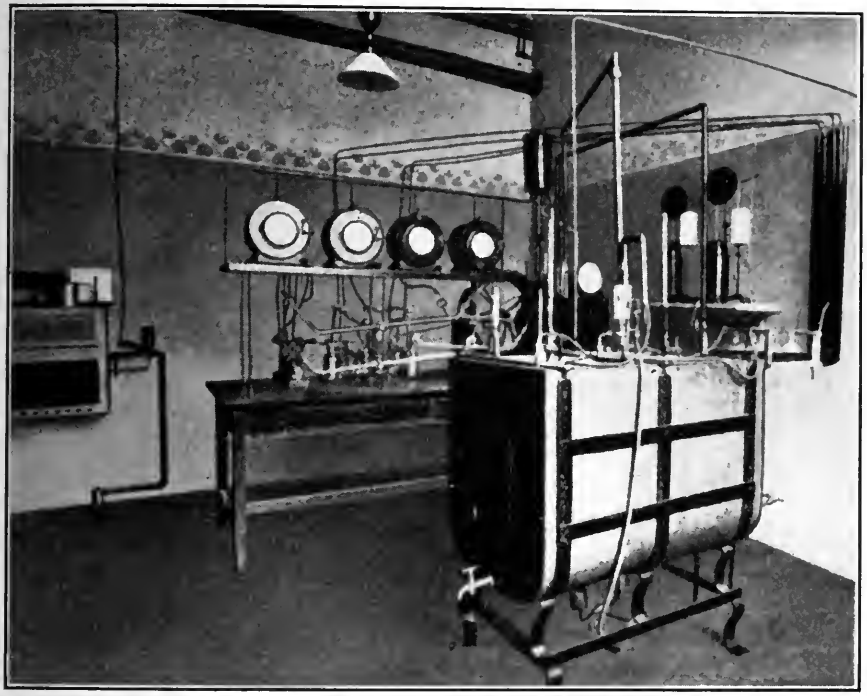


Fig. 31. The second calorimeter of Rubner. Description in the text.

back of the calorimeter). As a control mechanism another spirometer registers in the same manner the summated expansion of four vertical air-cushions in the four corners of the water bath isolated from the first air-cushion. Fluctuations due to variations of temperature from extraneous causes or to variations of barometric pressure are thereby controlled.

C. Basic Principles of Energy Metabolism

Only the most important generalizations concerning the energy metabolism in normal warm-blooded animals will be attempted here. While some of these are not yet universally accepted, sufficient evidence is at hand in the case of all of those which will be discussed to dignify them with the

designation of "basic principles." Some indeed are so fundamental and so universal in their application as to deserve the designation, "laws of metabolism." But it will avoid controversy to employ the more conservative term.

I. The Principle of the Conservation of Energy in the Animal Organism

Lavoisier, the father of metabolism, foresaw that the heat of the animal body could be measured by two means: the computation based upon the chemistry of combustion, and direct measurement (Gavarret), and it is almost certain that had he been permitted to complete his researches in this field the demonstration of complete agreement by the two methods would have lain to his credit. Without following the historical development of the subject or recording the failures which intervened we may pass at once to the work of Rubner(*d*). With the calorimeter just described Rubner studied the heat production of dogs by the two methods. He determined the C and N of the excreta and computed the amount of protein and fat metabolized in fasting and after feeding with meat and lard. Multiplying the protein and fat by the physiological heat values of these foodstuffs recently determined by him (page 551) he obtained the heat production by and indirect method. At the same time his calorimeter recorded the actual amount of heat eliminated. His results are given in Table 11.

TABLE 11

HEAT PRODUCTION OF DOGS BY DIRECT AND INDIRECT CALORIMETRY (Rubner)

No.	Animal	Food per Day	No. Days	Calories Heat Prod. Calculated	Calories Heat Prod. Measured	Difference in per Cent
1	Dog I	Fasting	5	1,296.3	1,305.2	0.69
2	Dog II	Fasting	2	1,091.2	1,056.6	— 3.15
3	Dog I	390 gm. meat	1	329.9	333.9	1.20
4	Dog I	40 gm. lard	5	1,510.1	1,495.3	— 0.97
5	Dog I	80 gm. meat 30 gm. lard	12	3,985.4	3,958.4	— 0.68
6	Dog I	same	8	2,492.4	2,488.0	— 0.17
7	Dog I	350 gm. meat	6	2,249.8	2,760.9	1.20
8	Dog II	580 gm. meat	7	4,780.8	4,769.3	— 0.24
			46	17,735.9	17,683.6	— 0.30

In a total of forty-six days of experimentation, with his animals Rubner thus found a difference of only 0.3 per cent between the heat production as calculated and the heat production as directly measured. This proves that the energy set free by oxidation (in the absence of external work), whatever transformations it may undergo in the body, finally leaves the body as heat. In other words, all the available energy which entered the body in potential form has been recovered as heat, and the applicability

of the law of the conservation of energy to the animal body was thus demonstrated.

Atwater and his colleagues, Rosa, Woods, Benedict, Smith and Bryant studied this balance of energy in a series of rest and work experiments by means of the Atwater-Rosa calorimeter (Atwater and Benedict(*a*, *b*)). On four different human subjects the agreement between the direct and indirect methods were almost as close as those reported by Rubner. The results may be summarized briefly as follows:

TABLE 12

HEAT PRODUCTION OF HUMAN SUBJECTS BY DIRECT AND INDIRECT CALORIMETRY (Atwater et al.)

	Heat as Calculated Cal.	Heat as Measured Cal.	Difference per Cent
Average of 67 days rest experiments	2258	2270	0.6
Average of 76 days work experiments	4567	4554	—0.3
Average of all experiments	3597	3577	—0.6

The results are perfectly clear-cut. The heat-production as calculated from the heat value of the food and from the heat value of the excreta (for method of calculation see page 552) agrees exactly with the amount of heat eliminated. The food in these experiments consisted of the three classes of foodstuffs and on certain days included alcohol in small amounts. The assumption was made (see page 554) that carbohydrate absorbed enters into combustion before the fat. The close agreement between direct and indirect measurement seems to justify the assumption.

All of the experiments thus far cited in support of the principle of the conservation of energy continued for 24 hours. We now know, however, that the principle holds for short periods as well. Thus Howland(*a*) working with the Cornell calorimeter found that with young children the heat production, expressed in calories per hour, as measured by the calorimeter differed from the heat production as calculated from the respiratory exchange and the nitrogen output, on six different days, by only 2.1 per cent.

With the same calorimeter Murlin and Lusk found in a series of twenty-two experiments in hourly periods on a dog, which was being fed large amounts of fat alternating with fasting periods, 2244 calories[5] by indirect calorimetry as against 2230 calories by direct calorimetry, a difference of 0.6 per cent. A large part of the energy was derived from the emulsified fat given for the most part without other food. These peculiar circumstances did not interfere in any way with the fundamental dynamic principle.

[5] Throughout this chapter the large calorie is not capitalized unless abbreviated as in Table 12. In human metabolism the large calorie is always understood unless otherwise designated.

Gephart and DuBois(*a*) in the first twenty experiments with the Sage calorimeter upon normal subjects, some of them in the post-absorptive state and others soon after taking foods of various kinds, reported a total heat production of 4577.37 calories by calculation as against 4569.4 by direct measurement, a discrepancy of only 0.17 per cent.

Instances might be multiplied further but it is unnecessary. The potential energy of the food in so far as it is oxidized is returned by the body without loss, in kinetic form; and even when measurable work is done the energy can all be accounted for.

II. The Energy of Muscular Work is Definitely Related to the Potential Energy of the Food

1. Origin in Non-Nitrogenous Food.—When Liebig had completed his classification of the foodstuffs, and had found that all animal tissues contained proteins, i. e., are nitrogenous, he suggested that the excretion of nitrogen by the animal might be used as a measure of protein destruction in the animal's body. Carl Voit, who had been a pupil of Liebig, was among the first to put this suggestion to practical use. Among many other important facts, regarding the metabolism of proteins, Voit discovered that, contrary to the teaching of Liebig, the protein of the body is not the source of the muscular energy; for, during muscular work, no more nitrogen is eliminated than in muscular rest. Since it had been known from the time of Lavoisier that muscular exercise increased the heat production, it followed, from the observations of Voit, that the non-nitrogenous foodstuffs must be the source of the extra heat production as well as of the energy of muscular contraction. This fact is now thoroughly established by almost numberless experiments (Lusk(*h*)). An illustration may be taken from the work of Atwater cited above. A subject doing work on the bicycle ergometer produced in twenty-four hours 5,100 calories of heat, of which 434 calories came from the protein ($N \times 6.25 \times 4.1$). In muscular rest this same individual produced 2,270 Calories, of which 400 came from protein. The day's work had increased the total heat production 2,830 Calories, but the heat from protein had been increased only thirty-four calories. All of the rest, 2,800 Calories (nearly), came from non-nitrogenous food.

2. Mechanical Efficiency of Muscular Work.—Soon after the law of the conservation of energy was enunciated by Mayer, the mechanical efficiency of muscular work done by a horse was computed by Joule. He showed that a horse could perform work equivalent to twenty-four million foot pounds in one day, during which time the food consisted of 12 pounds of hay and 12 pounds of corn. From original measurements of the heat value of this food Joule inferred that one grain of food consisting of equal

parts of undried hay and corn could raise one pound of water 0.682° F., which from previous experiments he knew was equivalent to 557 foot-pounds. From these results it appeared that one-quarter of the whole amount of energy generated by combustion of the food could be converted into useful mechanical work, the remaining three-quarters being required to keep up the animal heat, etc. (Scoresby and Joule).

Since these first measurements by Joule many estimates have been made of the mechanical efficiency of various kinds of muscular work both in animals and men. It turns out that the efficiency depends upon the type of work performed, i. e., the particular muscles used, the training, the speed with which the work is done, and the kind of food which sustains the metabolism.

It is necessary at this point to distinguish between gross efficiency and net efficiency. The former term is found by dividing the mechanical work in terms of heat by the *total metabolism* of the time; while net efficiency, the more exact term from the standpoint of bio-physics, is found by dividing the heat equivalent of the mechanical work by the *extra metabolism* due to the work accomplished. This is found of course by subtracting the basal or resting metabolism from the total work metabolism. Unless otherwise specified the figures used in this chapter refer to net efficiency.

From data obtained by Lavoisier upon his assistant, Séguin, whose oxygen absorption was measured during rest and while working a treddle, Benedict and Cathcart have calculated that at most an efficiency (net) of 7.7 per cent can be made out. This work of Lavoisier represents the earliest collection of data from which the efficiency of human muscles can be computed. Helmholtz presented the next in order historically when he assembled data from the work of Edward Smith, of Dulong and of Despretz, which according to his reckoning showed a gross efficiency of approximately 20 per cent. Amar cites experiments by Hirn done in 1857 which, assuming that the total heat elimination was correctly measured, demonstrate an efficiency of about the same amount. Other important workers of the French school in this field are Laulanié(*d*) and Chauveau(*a*). The former studied especially the influence of speed upon efficiency. He found in experiments upon himself that so long as the rate was constant, turning a wheel with a brake attachment 5, 10 or 15 minutes gave the same efficiency, but when the load and speed were varied the efficiency varied from 9 to 23 per cent. The load varied from 1 to 15 kilograms and the speed from 1.49 to 0.13 meter per second. The highest efficiency was shown with a moderate load (4 kilograms) and a moderate speed (0.61 meter per second). This accords with everyday experience.

Chauveau's observations made upon his assistant, Tissot, were directed especially to the question of the kind of foodstuffs which supports muscular work. They will be referred to later.

The German laboratories which have contributed most to the literature of mechanical efficiency in muscular work are those of N. Zuntz and of Kronecker. Both used the method of Zuntz in determining the respiratory exchange. Magnus-Levy(*g*), Durig (*c*), and Loewy (*a*), all of the Zuntz school of workers, have given important summaries of this work up to 1911. Durig's own experiments under Kronecker's direction, as well as those of Zuntz, and Loewy, Müller(*a*), Caspari(*a*), Zuntz and Schumburg(*a*), and L. Zuntz, show plainly the effect of training upon muscular efficiency, as well as the influence of velocity. Much of the work was done with the treadmill, some with an arm ergometer and other experiments in which the respiratory exchange was measured by means of the Zuntz portable apparatus was done in marching on roads or climbing mountain trails. The treadmill showed net efficiencies as high as 37 per cent, with the average at 31 per cent. The arm ergometer gave the lowest efficiency, namely, 19 per cent and the mountain climbing and marching experiments intermediate results. In certain experiments of the latter class carried out in summer upon a mountain trail which had an inclination of 16.4 per cent Durig's own efficiency was 31.1 per cent and that of his three companions was 30.3, 31.7 and 30.1 per cent respectively. In bicycle riding L. Zuntz, who was the first to make studies of the respiratory exchange in this type of work, found values which later were calculated to show a net efficiency of 28 per cent (Berg, DuBois-Reymond and Zuntz, L.). Benedict and Carpenter, using the same type of work but changing the bicycle to a stationary ergometer, found an average of only 21.5 per cent, a figure which has been substantially confirmed by a more recent and extensive study by Benedict and Cathcart.

The effect of training is shown in the following table from Benedict and Cathcart exhibiting the maximum gross and net efficiencies for their six subjects. The highest efficiency in both senses is shown by the one professional bicycle rider (M.A.M.) of the group.

TABLE 13

MAXIMUM GROSS AND NET EFFICIENCIES WITH THE BICYCLE ERGOMETER (Benedict and Cathcart)

Subject	Gross, per Cent	Net, per Cent
E. P. C.	19.9	23.1
J. J. C.	17.8	20.4
H. L. H.	18.6	21.6
J. E. F.	19.8	22.7
K. H. A.	18.2	20.8
M. A. M.	21.2	25.2

Benedict and Cathcart have also given attention to the relation of speed to muscular efficiency. They find that while in general the efficiency increases with the load (amperage of current actuating the brake) with

the heaviest loads there were definite indications of decreased efficiency. Figure 32 exhibits the relationship of total metabolism to effective work at varying speeds but with a constant load. In computing the net efficiency the basal metabolism obtained with the subject lying quietly on a couch was used and since this is practically constant, the net efficiency would be effected by speed in the same way as the gross efficiency (total heat output). The figure shows that in order to produce 1.565 calories of effective muscular work at 70 revolutions per minute it is necessary for the subject to produce a total of 7.61 calories (gross efficiency 20.6 per cent); while to produce 2.425 calories of work at 130 revolutions required 15.04 calories of heat (gross efficiency 16.1 per cent). "From the upper curve it is seen that the output of heat is constant per 10 revolutions; on the other hand, the increase in effective muscular work performed is not constant for each ten revolutions, but there is a distinct falling off. If, therefore, we divide the increase in the external muscular work between any two points on the curve by the increase in the total heat output corresponding to the same two points, we get an efficiency based upon increasing speed, the load being the same. For instance, in changing from 70 to 80 revolutions per minute, there is an increase in the effective muscular work equivalent to 0.205 calorie. Under these conditions there is an increase in the total heat output of 1.24 calories. Dividing the increase in heat output due to the muscular work (0.204 calorie) by the increase in the total heat output (1.24 calories) we find an efficiency for the increased amount of work performed of 16.53 per cent." Compu-

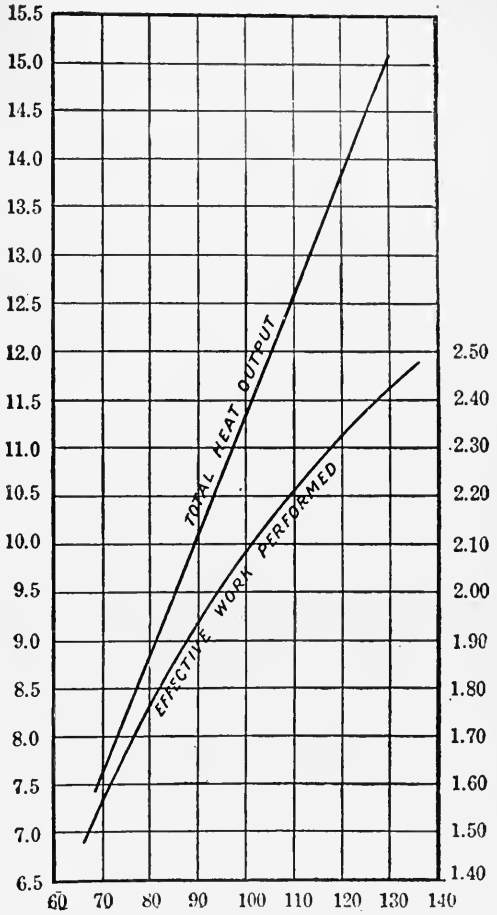


Fig. 32. Curves showing the total heat output per minute and corresponding external muscular work per minute, expressed in calories, for subject riding with constant load—1.5 amperes—at varying speeds. (Benedict and Cathcart.)

tations for the corresponding increase of ten revolutions gives from 90 to 100 revolutions 11.94 per cent, and from 120 to 130 revolutions 7.82 per cent, with intermediate values in percentage for the intervening increments. Net efficiency showed a similar falling off with the higher rates of speed. For example, when the effective muscular work was 1.95 calories per minute, at a rate of 90 revolutions the net efficiency was 22.6 per cent, while at 124 revolutions per minute it was only 15.7 per cent.

3. Relative Value of Different Foodstuffs as a Source of Energy in Muscular Work.—From his experiments upon Tissot as subject in climbing and descending stairs, Chauveau came to the conclusion from a consideration of the respiratory quotients, that carbohydrate alone furnishes the energy of muscular work and that fat can only be utilized by first undergoing transformation to carbohydrate. Zuntz and Heinemann, however, point out that if Chauveau's hypothesis of transformations were true, 30 per cent more energy for each unit of work performed should be liberated when fat burns than when carbohydrate is the starting point. Zuntz further criticizes Chauveau's experiments as being too extreme in severity (the subject was exhausted at the end of 70 minutes) and not of sufficient duration. Experiments by himself and associates in which precautions in both respects were carefully observed gave respiratory quotients during work which were exactly the same as in muscular rest. He cites especially the following results of Heinemann made with the Gärtner ergostat and the Zuntz respiration apparatus.

TABLE 14

ENERGY PRODUCTION OF MUSCULAR WORK ON DIFFERENT DIETS (Heinemann)

Food	Rest		Work		Amount of Work, Kgm.	Per Kgm. of Work	
	O_2 per Min., c.c.	R. Q.	O_2 per Min., c.c.	R. Q.		O_2 c.c.	Cal.
Fat	319	0.72	1029	0.72	354	2.01	9.39
Carbohydrate.	277	0.90	1029	0.90	346	2.17	10.41
Protein	306	0.80	1127	0.80	345	2.38	11.35

It appears from this comparison that there really is little difference between fat and carbohydrate, and that protein likewise as the chief constituent of a diet occupies a place only a little less favorable as a source of muscular energy. The respiratory quotients were the same for each foodstuff during muscular work as during rest.

This last statement seems to be true, however, only with the moderate intensity of work which Zuntz observed. Benedict and Cathcart found the average respiratory quotients with their professional bicycle rider were as follows:

	Before Work	During Work	After Work
16 days moderate work	0.84	0.84	0.77
16 days heavy work	0.85	0.90	0.78

Brezina and Kolmer likewise noted that the height of the initial respiratory quotients during periods of muscular work varied with the intensity of the work performed. When 1.6 calories per minute was the rate of metabolism the R. Q. was 0.83; but when the rate rose to 10 calories per minute the quotient was 0.99. Lusk, who quotes this experiment, explains the higher quotients as due in part to the formation of acid with consequent liberation of CO_2 from the plasma more rapidly than it was formed. Other factors, he states, are the increased ventilation of the lungs and carbohydrate utilization; for acid formation accelerates the conversion of glycogen to glucose. In very extreme work, especially in short spurts, it is quite possible also that oxygen absorption does not quite keep pace with CO_2 elimination from the lungs. Hence the purplish color of the face in muscular exhaustion as contrasted with the lighter but healthier color of moderate exercise. After exercise when the oxygen absorption is gaining on the CO_2 elimination the tendency would be for the R. Q. to be depressed. That there is a real and not an imaginary mobilization of carbohydrate during work Benedict and Cathcart infer from the fact that following carbohydrate-rich diets the quotient rises somewhat more in work than it does following carbohydrate-poor diets.

As regards the mechanical efficiency upon different diets Zuntz was convinced that there was nothing to choose between carbohydrate and fat. He cites experiments performed by his students, especially Frentzel and Reach and also of Atwater and his colleagues, which show that the absorption of oxygen is essentially the same whether carbohydrate or fat is burned (see Table 14). Benedict and Cathcart support this view with their findings that the energy quotient (total calories produced per calorie of effective work performed) was the same on days following a carbohydrate-rich diet as on days following a diet poor in this foodstuff whether the amount of work was large or small. Anderson and Lusk performed experiments upon a 9 kilo dog while running upon a treadmill inside the calorimeter both before and after feeding with large amounts of glucose and noted a distinct difference in efficiency after the carbohydrate ingestion. When the dog had been without food for 18 hours and the average respiratory quotient was 0.78 it required 0.580 kilogrammeter of work to move 1 kilo of the body weight 1 meter on the horizontal. In the first hours after carbohydrate when the average quotient was 0.95 the same work was done at an expenditure of 0.550 kilogrammeter, a saving of 5 per cent. Krogh and Lindhard point out that if the metabolism per unit of work is assumed to be a straight line function of the quotient the

waste of energy from fat in these experiments works out as eight per cent.

The last-named authors have carried the comparison between fat and carbohydrate as a source of muscular work much farther. They devised experiments upon human subjects with the bicycle ergometer of Krogh placed inside a Jaquet-Grafe (page 520) respiration chamber, which would be done, after the manner of Benedict and Cathcart's experiments, before the first meal of the day, but following two or more days upon controlled diets containing in turn a decided preponderance of the two non-nitrogenous foodstuffs. The two most successful subjects were college athletes familiar with bicycling, and, in one series, freshly trained. Both these students and three out of five older subjects experienced great difficulty in doing the prescribed work and suffered much fatigue thereafter following heavy fat feeding, but did the work with ease and without fatigue following carbohydrate. This experience accords with that of other observers.

The results of Krogh and Lindhard are summarized below.

TABLE 15

COMPARISON OF FAT AND CARBOHYDRATE AS SOURCE OF MUSCULAR ENERGY
(Krogh and Lindhard)

Subject	Calories per Unit Work		Difference		No. of Exp.	Average Net Efficiency
	From Fat	From Carbohy.	Cal.	Per Cent		
J. L.	5.69	4.59	1.10	19.4	10	
G. L.	5.84	5.09	0.75	12.8	15	18.3
A. K.	5.04	4.28	0.76	15.1	15	21.6
R. E.	4.72	3.72	1.00	21.2	13	23.7
M. N. Tb. XII .	4.70	4.02	0.68	14.5	33	23.0
M. N. Tb. XIII .	4.73	4.10	0.63	13.3	18	22.7
O. H. Tb. IX ...	4.79	4.32	0.47	9.8	33	22.0
O. H. Tb. XVI .	4.52	4.10	0.42	9.3	49	23.2
O. H. Tb. XVII .	4.52	4.15	0.42	9.2	24	23.0

The simple average of the percentage differences, the authors state, would be very misleading partly because of the different number of experiments for the different subjects and partly because the several series are by no means equally concordant. By assigning definite "weights" to each series in proportion to the number of determinations and in inverse ratio to the standard deviations within each series the average percentage waste of energy from fat as compared with carbohydrate is 11.25. It follows clearly that work is more economically performed on carbohydrate than on fat.

From the table it may be seen that the net expenditure of energy necessary to perform one calorie of mechanical work on the ergometer varies

between about 5.5 and 4.0 Cal. At a constant quotient the authors find that it varies somewhat with the subject, and for the same subject it decreases with training (see page 588).

The question may fairly be raised, Where does protein stand in the scale of efficiency as a source of muscular work? This question has been studied in relation to the specific dynamic action of protein by Rubner(*o*) and more recently by Anderson and Lusk. Both sets of observations show that there is practically complete summation of the extra energy production due to the specific dynamic action of meat and the energy production caused by the muscular work. There is nothing specifically uneconomical in doing work on a high protein diet except in the sense that the extra heat of dynamic action is added to the extra heat of muscular work and this throws extra burdens on the organs charged with the dissipation of heat. With cane sugar, as proved in Rubner's experiments or glucose as proved in Lusk's, the specific dynamic effect of the food disappears, i. e., merges into, the extra metabolism of muscular work. These facts make it clear that the mechanism of energy release in muscular work is more nearly akin to the mechanism by which carbohydrate raises the metabolism (metabolism of plethora, see page 606) than it is to the mechanism of protein stimulation. The work of Fletcher and Hopkins and of A. V. Hill on the details of muscular contraction make it appear that certain reactions take place between definite substances which must be closely allied to carbohydrates. It becomes more intelligible therefore why carbohydrate should support muscular work more economically than fat[6] and why its dynamic action, unlike that of protein, should not be superimposed upon the metabolism of muscular work.

III. The Energy Metabolism is Determined in Part by the Environing Temperature

1. How Heat is Lost from the Body.—In general, there are four main avenues of escape for the heat which is produced in the body of a warm-blooded animal: (1) Warming the food and air which enter the body; (2) Vaporization of water and setting free of CO_2 in the lungs; (3) Evaporation of water from the surface of the body; (4) Radiation and conduction from the surface of the body.

Tigerstedt(*a*) gives the following calculations made by Rubner for a man producing 2,700 calories daily:

[6] Krogh and Lindhard note that the standard metabolism (called basal metabolism more commonly) is somewhat higher when the respiratory quotient is low than when it lies in the median range. There is just a hint in this fact that the so-called waste of energy when muscular work is supported by fat may be bound up with the specific dynamic action of that foodstuff as it is in the case of protein.

	Calories
(1) Warming food and drink to body temperature	42
(2) Warming air from 17.5° to 30° C.	35
(3) Evaporation of water from lungs and skin	558
(4) Heat equivalent of external work done	51
(5) Loss of radiation from entire surface of body	1,181
(6) Loss by conduction to air from entire surface	833
Total	2,700

Atwater, in his calorimetric studies, made the following estimations:

I. Resting man, mean of fourteen experiments comprising forty-two days:

	Calories
1. Heat loss by radiation and conduction	1,683
2. Heat loss by urine and feces	31
3. Heat loss by evaporation from lungs and skin	548
Total	2,262

II. Man at work, mean of twenty experiments comprising sixty-six days:

	Calories
1. Heat loss by radiation and conduction	3,340
2. Heat loss by urine and feces	46
3. Heat loss by evaporation from lungs and skin	859
4. Heat equivalent of muscular work	451
Total	4,676

It is evident, from these estimates, that fully eighty per cent of all the heat produced in the body is lost through the skin.

2. The Law of Surface Area.—Closely related to this matter of the loss of heat through the skin is the relationship of heat loss to heat production known as the law of surface area, first enunciated over 80 years ago by certain French writers. To quote one of the earliest communications: "As the heat loss is proportional to the extent of free surfaces and these latter are to each other as the squares of their homologous sides, it follows of necessity that the quantity of oxygen absorbed, or what amounts to the same thing, the heat produced on the one hand and lost on the other, is proportional to the square of the corresponding dimensions of the animals one is comparing (Robiquet and Thillayé)." The first experimental evidence of relationship between skin surface and the food requirement of animals seems to have been furnished by Müntz who in 1879 investigated the maintenance ration of horses. Emphasizing the part played by the surface he says: "A notable part of the food certainly is consumed to maintain the vital heat which has a tendency constantly to be lost by radiation or conduction to the surrounding medium. Another cause of cooling is cutaneous evaporation which is a function of the surface if it is not directly proportional thereto. The evaporation produced by the organs of respiration may equally be regarded as having a relation to the surface of the body rather than to the weight. We are then by these considerations in position

to admit the preponderating influence of surface upon the apportionment of the maintenance ration."

This law of surface a few years later was placed upon a firmer basis by researches of Rubner(*a*) upon dogs and of Richet(*c*) upon rabbits.

A small animal has a greater surface, in proportion to its weight, than has a large animal. This will be clear from the following illustration. Suppose we have two spheres of two and four centimeters diameter. The surface of the smaller would be 12.56 square centimeters and of the larger 50.24 square centimeters. The volume of the first would be 4.18 c.c. and of the latter 33.49 c.c. The surface of the smaller, in proportion to its volume, therefore, would be as 3:1, while of the larger it would be only as 1.5:1. Since, now, more than four-fifths of the animal's heat escapes through the skin, by one physical means or another, it is clear that heat must be produced in proportion to the surface rather than in proportion to the mass, if the body temperature is to be maintained. Hence, if two animals, with similar coats of fur, had skin surfaces that bore to each other the relation of these spheres, the smaller animal would produce twice as much heat per unit of weight as the larger. Rubner found that the average heat production per square meter of body surface for man, dog, rabbit, guinea pig, and mouse was 1,088 calories with variations of + 104 calories to — 103 calories, i. e., of about ten per cent either way.

a. *Measurement of the Surface Area.*—Several methods have been proposed for determining the surface area of the human subject. The first was that of Mech who marked out some parts of the body, which were favorable for the purpose, in geometrical figures, covered them with transparent paper and made tracings of the figures. The areas of these figures were then calculated or determined by weighing the paper. Other parts of the body were measured directly by wrapping with millimeter paper. Bouchard suggested a plan which was later improved upon by DuBois and DuBois(*a*), namely, of clothing the body in tights made of some thin inelastic material which could be weighed. D'Arsonval(*c*) clothed a man in silk tights and after charging the clothing with electricity, determined the surface relative to a metal plate of known surface by releasing the charge as from a Leyden jar. Lissauer measured the surface of dead infants by covering the skin with adhesive material, applying silk paper, and then measuring the area of the paper by means of a planimeter.

The measurement was accomplished by DuBois in the following manner. A light, flexible, inelastic covering was obtained by clothing the body with a close-fitting knitted union suit, and pasting this over with adhesive paper. But instead of attempting to weigh this "model" of the body surface, it was cut up into pieces which would lie out flat and the area of each piece determined by photographing it on sensitive paper. The total area was then found by weighing the photographic silhouettes and comparing with the weight of a unit area of the same sensitive paper.

The areas of the several members of the body as measured were then compared with the areas as given by multiplying their lengths by sums of measurements representing circumferences. For example, the area of the arm was given by multiplying the length from the outer end of the clavicle to the lower border of the radius (F) by the sum of the three circumferences at: upper border of axilla (G); largest girth of forearm (H); smallest girth of wrist (I). This calculated area compared with the actual area for several individuals gave a factor which, used with the product first given, made up a so-called linear formula for the arm; thus: F (G + H + I) 0.558. The several sub-formulæ added together could then be employed for measuring the surface of the entire body.

This method resembles the one proposed by Roussy in which the surface

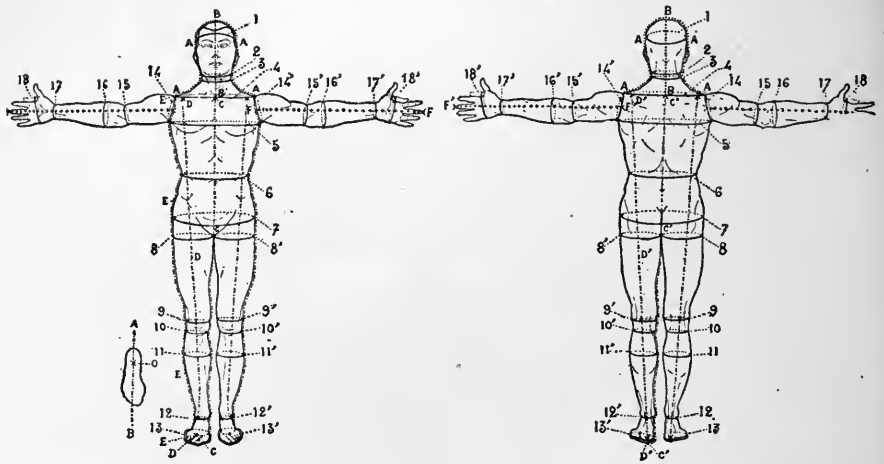


Fig. 33. A method of calculating the surface area by treating the body as a series of cylinders. The average is taken of 29 different circumferences (mean perimeter) and this is multiplied by the sum of the several lengths. (Roussy.)

was given by multiplying the mean perimeter (Pm) by the mean peripheral total height (Hm); thus S = Pm × Hm. The first factor was found by taking the mean of 29 different circumferences (Fig. 33) while Hm is the sum of 3 partial heights, (a) head, neck and shoulders; (b) trunk and lower extremities; (c) upper extremities.

From his measurements Meeh devised a formula based upon the well known relationship of surfaces to masses of similar solids; namely, that the former varies as the ⅔ power of the latter. By employing a constant, 12.3, Meeh found that the formula $S = \sqrt[3]{(w)^2}$ gave results within 7 per cent of those determined by actual measurement. DuBois found an agreement between measured and calculated values for 5 cases within 2 per cent. Later his measurements were simplified and a formula containing total height, weight and certain constant factors was devised. This is known as the weight-height formula. $A = W^{0.425} \times H^{0.725} \times C.$,

where A is the area in sq. cm., H the height in centimeters, W the weight in kgm., and C a constant 71.84. A chart based upon this formula for direct reading of the surface area when height and weight in metric units are known is given in Fig. 33-a.

b. *Criticisms of the Law of Surface Area.*—Various criticisms have been leveled at the law of surface area, some of them based upon fact and some upon interpretation. Of the criticisms based upon fact that recently published by Harris and Benedict is perhaps the most important. They have subjected the body surface law to a critical biometric study and have reached the conclusion that the correlations between body surface and basal heat production in normal adults are of about the same magnitude as those between body weight and heat production. "These results do not, therefore, justify the conclusion that metabolism is proportional to body surface and not proportional to body weight." In the opinion of these authors the closer agreement between heat production of different indi-

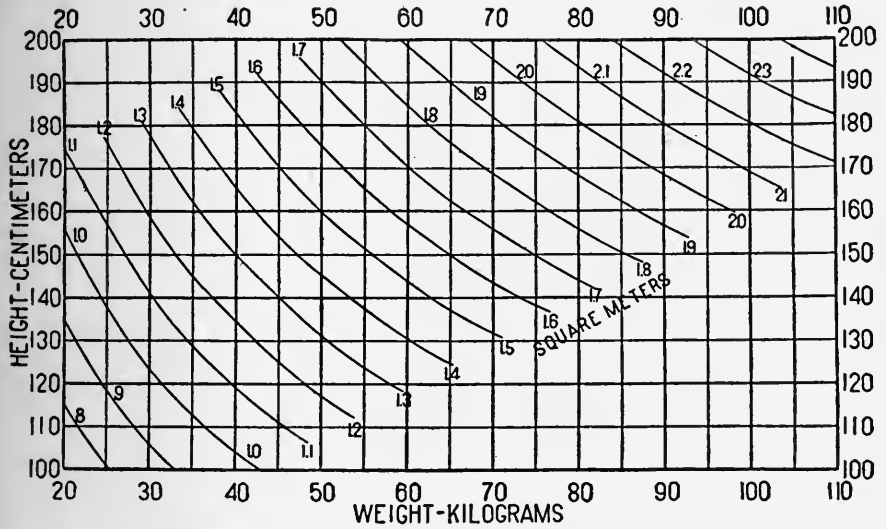


Fig. 33-a. Chart for determining surface area of man in square meters from weight in kilograms (Wt.) and height in centimeters (Ht.) according to the formula: Area (Sq. M.) $= Wt^{0.425} \times Ht^{0.725} \times 71.84$ (DuBois).

viduals and their surfaces than between heat production and body weight is not due to any causal relation between heat loss and heat production as a mechanism for preservation of heat loss and body temperature, but in part at least proceeds from the fact that body surface being proportional to the ⅔ power of weight is less variable than the weight itself, and the ratio of heat produced to body surface consequently is likewise less variable.

As a matter of fact the mathematical relationship does not stop here; for in many instances the constant employed in the formula, for example,

of Meeh or of Lissauer by which the ⅔ power of the weight is multiplied equalizes the proportions between surfaces and weights. This fact gives a slightly different posture to the argument. A few illustrations will make this clear. Suppose, for example, we have two infants weighing 7 and 8 kilograms respectively. Expressing their weights in kilograms and their surfaces in sq. M. by the Meeh and Lissauer formulas, we have the proportions shown in the following table.

TABLE 16

Relation of Body Weights and Surfaces to Each Other

Weight, kgm.	Ratio	Meeh-Rubner $11.9\sqrt[3]{(w)^2}$ Surface, sq. M.	Ratio	Lissauer $10.3\sqrt[3]{(w)^2}$ Surface, sq. M.	Ratio
7		0.4353		0.3769	
8	0.88	0.4760	0.91	0.4120	0.91
20		0.8768		0.7589	
21	0.95	0.9058	0.97	0.7840	0.97
40		1.3920		1.205	
41	0.98	1.4150	0.98	1.225	0.98
4		0.299		0.259	
40	0.10	1.3920	0.210	1.205	0.21
3.5		0.274		0.237	
70	0.05	2.021	0.135	1.750	0.136

The ratio of weights is .88 : 1 and of surfaces .91 : 1. Now it is obvious that if the metabolism of these two children is proportional to their weights it must of necessity also be nearly proportional to surface. With two youths weighing 40 and 41 kilos the surfaces bear to each other exactly the same ratio as the weights, whether the Meeh or Lissauer formula be employed. Both, therefore, will be equally good measures of metabolism for the two individuals.

Contrast with this the relationship between individuals weighing 4 and 40 kilograms, or still better, an infant at birth weighing 3½ kilograms and a man weighing 70 kilograms. In the latter the weights are to each other as .05 to 1, and the surfaces as .135 to 1. In other words, the weight of the larger individual is twenty times that of the smaller, while the surface is a little over seven times that of the smaller. In this case weight and surface cannot possibly be of equal value as measures of the metabolism. One is nearly three times as good—or as bad—as the other. As a matter of fact it is now well known that surface is about two and one-half times as good a measure as weight between two such individuals.

Benedict and his colleagues have fallen into the error of supposing that physiologists have believed the basal metabolism to be absolutely proportional to surface regardless of circumstances. This is quite incorrect. Rubner for the German literature and Richet for the French are respon-

sible for the first demonstrations of the applicability of the law. Rubner worked with dogs of adult stature but widely different size, estimating their metabolism by the indirect method. Richet worked first with rabbits ranging from 2000 to 3500 grams in weight but he determined only the heat of radiation and conduction, neglecting, as nearly all subsequent French observers have done, the heat given off by evaporation. Naturally his quantities would be more nearly proportional to surface than the total. However, in the estimation of surfaces he says, "If one supposes that animals of different size are like spheres of different volumes, then the respective volumes are related among themselves as the cubes of their radii; while the respective surfaces are related among themselves as the squares of their radii. These considerations apply to living animals, and, since their form is so irregular compared with that of a perfect sphere, one can only apply the geometrical facts to them approximately." Further in summing up the factors which determine heat production Richet notes that one of these is "the nature of the integument." In two important respects, therefore, Richet made saving clauses regarding the application of the law of surface, one concerning the measurement of surface and the other concerning the nature of the skin, meaning, of course, its conducting properties. Rubner in the beginning considered that he had demonstrated the law only for adult animals and later in applying it to children made this very emphatic reservation: "The law of surface area holds under all physiological conditions of life, but for its proof it is a reasonable presumption that only organisms of similar physiological capacities, as regards nutrition, climatic influences, temperament, and functional power, should be compared." Other students of metabolism have made similar reservations. Thus Schlossmann says, "The presumption is on the one hand that the environment is relatively normal, on the other that the child has a relatively normal surface, that is, a functioning and good conducting skin with the normal amount of subcutaneous fat." Otherwise, he thinks, the law could not be expected to apply.

The arguments against the law, so far as they rest upon facts, seem, as we have just seen, to have been misconceived. It never was supposed by its chief proponents that the law would apply to all physiological and pathological conditions but only to similar physiological (normal) conditions. Also, a very superficial understanding of the necessary mathematical relations shows that the law has natural limitations which must be recognized if one is to avoid compromising it with impossible conditions.

There is no doubt that Rubner, following Bergmann, has conceived of the law as causally related to Newton's law of cooling. This dependence as commonly accepted may be phrased in this way. Solid bodies when warmed lose heat in proportion to the difference between the temperature of the body and the temperature of the surrounding medium. Since this

heat must all pass through the surface it follows, other things equal, that they will lose heat for any particular gradient of temperature in proportion to surface. As applied to the animal body it is observed that the body temperature is *nearly* constant. Hence, if heat is lost in proportion to surface, it must also be produced in proportion to surface. This implies a causal relationship between surface loss and interior production of heat. An elaborate biometric analysis proves nothing more regarding this causal relationship than is proved by the simple mathematical analysis shown in Table 16. Whatever the physiological measurement of surface, if it can be expressed even approximately by a formula such as Meeh's it will follow that the ratio of body weights for certain ranges will be the same as the ratio of body surfaces *provided the weights are not far apart,* and for subjects of a continuous series in which weights differ by small increments it will follow that surface will be only a little, if any, better as a measure of metabolism than weight.

The question of causal relationship stands just where it always has stood. If the possession of a large surface in proportion to weight, as in a mouse, is accompanied by a vastly higher heat production per unit of weight as compared with a horse, but the heat production is found to be proportional to the surfaces in two such animals with approximately the same body temperature, it seems to follow that surface loss of heat is at least a more probable *cause* of heat production than body mass. The same is true as between a baby and a man.

On the basis of interpretation the objections to the law of surface run in this way. Since the heat production of animals seems to be proportional to surface area, it would seem to follow that heat is produced *in order to* replace that which is lost, or *to maintain* body temperature. This view, some say, denotes an all too naïve conception of nature. Blood does not coagulate in order to prevent hemorrhage, but because certain chemical agents are present with certain properties. The fact that it does stop hemorrhage is quite incidental. It may have selective value, so that a species whose blood did not clot would have the worst of it in the struggle for existence, but it will never do to say that this chemical-physiological function originated for the purpose of preventing hemorrhage; for that would imply a mind at work in anticipation of the result. So also with heat production. These critics, of whom Kassowitz(*c*) has been chief, prefer to account for heat production in a perfectly causal manner. "Small animals maintain a higher rate of oxidation, it is true, than large ones, but this is not because they lose heat more rapidly in consequence of greater (relative) surface, but because their alternating movements (later phases caused reflexly by earlier phases) follow one another more rapidly on account of shorter nerve paths." Kassowitz(*d*) indeed finds that the higher rate of oxidation in small, warm-blooded animals has even for them "dysteleological consequences; for because of the more extensive muscular con-

tractions more food and reserve substances are placed in requisition and by this means the deposit of reserve fat in the whole body, and especially in the subcutaneous tissues, is made more difficult, so that the protection against cooling—which a thick layer of fat prevents—fails in part amongst the very animals which need it most." Even Kassowitz is obliged to admit, however, that "in warm-blooded animals which are in a position to maintain their own body temperature under the most diverse conditions, one can claim the appearance of some justification that their living parts produce heat in order to protect the body against loss by radiation, etc."

Whether this is a real justification or only the appearance of one will not trouble the practical physiologist so long as the generalization that human beings of different size produce heat in proportion to surface rather than weight, and therefore, require food energy in proportion, helps him to understand his feeding problems; and there is no doubt that the law of surface area has been immensely useful in this connection. It explains the much higher basal metabolism per unit of weight of the small individual in comparison with the large, better than the so-called causal explanation cited by Kassowitz. It explains also much better the need for conservation of heat in the infant, and the rôle which subcutaneous fat plays in this connection.

3. Heat Production as Affected by External Temperature.—*a. In Cold-blooded Animals—Van't Hoff's Law.*—Increased activity in living tissues is almost invariably accompanied by an increased evolution of heat. Since this heat is derived from the chemical changes which proceed in the living cells, and since all chemical processes are quickened by a rise of temperature, we should expect to find that the heat produced in the metabolic processes of the organism would tend of itself to quicken these processes. This is found in fact to be the case. In most chemical reactions a rise of 10° C. would increase the velocity of the reaction from two and a half to three times (Van't Hoff's Law), and the same law is, within the limits of the stability of living tissues, found to apply to the process of oxidation. For example, in the early growth of a lupine seedling it has been found that the output of CO_2 bears to the temperature the following relationship:

0° C.	6	milligrams per hour
10° C.	18	" " "
20° C.	44	" " "
30° C.	86	" " "

The same relationship has been found to obtain for the production of CO_2 in the snail, the leech, and the earthworm. Perhaps the absorption of oxygen is a still better measure of the heat production. Within the range of 5 to 21° C. it has been observed that the factor (Q_{10}), which in biological literature expresses the number of times the process is accelerated for a rise of 10°, has, for the absorption of oxygen by the crayfish, a value of

2.5 to 3.5. In the case of the leech, the same factor, between 10 and 24°, is from 2.4 to 3.0 (Pütter, A.).

In living things the range within which any such law applies is necessarily very narrow as compared with its range in inorganic reactions; and the factor (Q_{10}) varies, according to the best determinations which have yet been made, very widely. Nevertheless, it may be said that the law that the rate of chemical change (metabolism) varies with the temperature of the living substance is a universal law for all animals and plants. As applied to the production of heat in living things, this law would result in a vicious circle (the temperature increasing the oxidation and the oxidation increasing the temperature) which would rapidly destroy the living substance itself, if special mechanisms did not exist for the removal of the heat. Where these mechanisms break down, as in fevers, the heat must be removed by artificial means.

DuBois(*b*) has recently shown that the metabolism of men in fevers increases from 30 to 60 per cent for a rise of three degrees (from 37 to 40° C.) and the value of Q_{10} therefore is about 2.3. In other words the metabolism in fevers obeys Van't Hoff's law.

b. *In Warm-blooded Animals.*—In warm-blooded animals with the development of the capacity to regulate the body temperature independently of the surrounding medium, Van't Hoff's law is apparently reversed, so that the lower the *external* temperature becomes the greater is the heat production. This is necessarily the case if the body temperature is to be maintained. Confirming the original observation of Lavoisier that more heat is produced in the human subject when the external temperature is low, C. Voit(*e*) exposed a man in light clothing in his respiration apparatus to different temperatures and found that, as the temperature fell, the metabolism increased independently of any muscular motions. Rubner(*h*) carried this line of investigation much farther, using dogs and guinea pigs, and formulated his laws of the chemical and physical regulation of the body temperature. In brief, these laws are : (1) That, from a temperature of about 30° C. downward, the body temperature is regulated chiefly by varying the heat production (chemical regulation). Heat loss is regulated, to some extent, by decreasing the amount of blood brought to the surface. (2) From 30° C. upward the body temperature is regulated chiefly by varying the amount of water evaporated from the surface (sweating) and again by decreasing the amount of blood brought to the surface (physical regulation).

The conclusions of Voit and Rubner with regard to the effect of cold as such have frequently been called in question, the contention being that even if visible shivering and increased tonus of the muscles are avoided no more heat is produced at low temperature. Lusk(*b*) found that a man immersed for a few minutes in a cold bath at 8° C. would, immediately thereafter, shiver enough to increase his metabolism 180 per cent above the

normal. Loewy(*c*) and Johansson conducted carefully controlled respiration experiments by two different methods with a view to the determination of the pure effect of cold. The former employed sixteen different subjects, cooling the body not only by exposure to a temperature of 12 to 16° C., but also by evaporation of water, alcohol and ether from the skin. The latter performed experiments upon himself as subject after acquiring the power to suppress all shivering or even increased tonus, when the naked body was exposed to a room temperature of 13 to 20° C. Both observers found that there was no increase in the elimination of carbon dioxid when the muscular factor was really ruled out. Uncontrolled shivering in Loewy's experiments produced an increase of 100 per cent in the metabolism.

Lefèvre(*d*) has demonstrated that the loss of heat from the skin does not follow Newton's law of cooling exactly because of certain physiological adjustments of which the skin and subjacent structures are capable. Nevertheless a better estimate of the influence of the environing temperature can be obtained by measuring the cooling power of the environment on a surface at body temperature than is given by a record of the outside temperature alone. The recognition of this truth led Leonard Hill(*b*) to invent an instrument known as the "Kata-thermometer." This consists of a large-bulbed spirit thermometer which is warmed up until the meniscus rises above 100° F. The rate of cooling is then determined with a stop-watch as the meniscus falls from 100° F. to 95° F. The constants of the instrument are determined, from which the cooling can be expressed in mille-calories ($\frac{1}{1000}$ grm. calories) per sq. cm. of surface per second. The instrument when used dry gives the rate of cooling by convection and radiation and when used wet (covered with a damp muslin glove) gives the rate of cooling by convection, radiation and evaporation. From the readings of the dry instrument can be deduced the velocity of movement of the dry air. The *evaporative* cooling power of the wet instrument depends on absolute humidity and wind.

Comparisons made by Hill between the rate of cooling of the Kata-thermometer with that of the naked pig as determined by Lefèvre and of the naked surface of the human forearm as determined by Waller, and with the dryness or sweating of the skin of soldiers producing a known amount of heat, suggests that the Kata-thermometer in air cools about three to five times as quickly as the naked skin when the temperature of the skin approximates closely to the body temperature.

Ordinary light clothes reduce the cooling power of the atmosphere of a man as well as of the instrument to one-half its value when unclothed.

The cooling power by radiation and convection exerted on the surface of the dry Kata-thermometer at 36.5° C. in mille-calories per sq. cm. per second according to Hill is as follows.

TABLE 17

COOLING POWER OF AIR CURRENTS AT DIFFERENT VELOCITIES (Hill)

Temp. ° Cent.	9 M. per Sec., 20 mi. per Hr.	4 M. per Sec., 8.8 mi. per Hr.	1 M. per Sec., 2.2 mi. per Hr.	½ M. per Sec., 1.1 mi. per Hr.	Still Air.
0	49.3 mille-cal.	36.1 mille-cal.	23.1 mille-cal.	19.0 mille-cal.	9.8
5	42.5	31.2	19.8	16.4	8.5
10	35.0	26.2	16.7	13.8	7.1
15	29.0	21.3	13.5	11.2	5.8
20	22.3	16.3	10.4	8.6	4.4
25	15.5	11.4	7.2	6.0	3.1

Flack and Hill made observations on the respiratory metabolism of several students by the Doublas-bag method (p. 537) and found that the heat production as calculated by the Zuntz-Schumberg method (p. 565) increased in different subjects from 27 to 82 per cent when they were sitting quietly on the roof of the laboratory, over the metabolism shown in the laboratory in the same clothing. For example, in one instance the heat production was 1.57 calories per minute in the laboratory and 3.12 Cal. in a strong cold wind on a snowy day. In another instance exposure to the inclement cold winds of an April (1918) day increased the resting metabolism of a young woman from 37 to 65 calories per sq. M. of body surface per hour.

Lefèvre had a subject who while lying on a bed naked, in an air current at 5° C. and of 1-2 meter per second velocity, for 3½ hours, exhibited a heat loss of 3 Cal. per minute as contrasted with 1.55 calories at 20° C. Sitting quietly in ordinary light clothes a man gave the following records of heat loss in air currents of 3.5 and 1 M. per second.

TABLE 18

Temperature	Weight, 65 Kg.	Surface 19,000 Sq. Cm.
	Wind Velocity 3.5 M. per Sec.	Wind Velocity 1 M. per Sec.
	Cal. per Diem.	Cal. per Diem.
— 1°	6,654	5,400
5°	4,704	4,000
10°	3,690	3,060
15°	3,144	2,317
20°	2,754	1,896
26°	2,270	

IV. The Ingestion of Food Increases the Metabolism

The observation of Lavoisier that the heat production was increased by taking food was confirmed by Pettenkofer and Voit(*b*), who found that the total metabolism of a dog was increased from 34.9 to 65 calories per kilogram as the result of eating about two and one-half pounds of

meat. Feeding fat they observed no increase in the heat production unless the amount fed was far in excess of the body requirements. Feeding carbohydrate in the form of starch, they found that 379 grams in the food increased the metabolism 17 per cent over that of the starving animal. More exact information concerning the influence of carbohydrate came with the invention of methods by Zuntz and by Benedict by which the oxygen absorption could be determined, since, without this knowledge, it was impossible to distinguish the part taken by fat in the total heat production from that taken by carbohydrates. Magnus-Levy, using the Zuntz method with human subjects, came to the conclusion, substantially in accord with those of Pettenkofer and Voit, namely, that moderate quantities of fat do not increase the heat production (absorption of oxygen), but that both carbohydrate and protein increase it considerably. Rubner, using only the excretion of CO_2 as the measure of heat production, formulated laws regarding the influence of different foods given to dogs, as follows: Since the different foodstuffs affect the heat production to a different degree, we may speak of their "specific dynamic action." The proper basis of comparison is the amount of heat produced by the fasting animal. Taking this quantity as the minimal requirement of the animal for energy (in potential form), and feeding this quantity in the form of different foodstuffs, the effect is for protein an increase of heat production of 30 per cent, for fat 11 per cent, for carbohydrate 5.8 per cent. In order to keep the animal in an energy equilibrium, therefore, it is necessary to feed him in protein 140 per cent of the requirement, in fat 114 per cent, and in carbohydrate 106 per cent.

Lusk and his co-workers, using the small respiration calorimeter (described on page 579), have demonstrated that the increased heat production in dogs after ingestion of proteins is due to the amino-acids into which the protein is broken up by digestion. It is, however, not the mere absorption of the amino-acids themselves, nor their direct oxidation which accelerates the metabolism, but the stimulating effect of the intermediate oxyacids which are formed from them. Quantitatively the results of these more modern researches confirm the conclusions of Rubner as to the specific effect of protein. These, however, relate to the dog. In man the dynamic effect is ordinarily not so great. The dynamic effect of protein in milk upon the metabolism of the infant will be discussed later (page 644). It need only be added here that protein which becomes a part of the body does not affect the heat production.

The dynamic effect of fat, it turns out, is not so high as Rubner found it, if reckoned for the entire day, but for individual periods up to six hours after feeding, may increase the metabolism as much as 30 per cent (Murlin and Lusk), as contrasted with protein (meat) which may raise it 100 per cent. Bloor found that the fat in the blood also increases up to six hours after feeding.

Following Rubner's fundamental observation on the influence of carbohydrate on the respiratory metabolism of a fasting dog, Magnus-Levy, Johansson, Durig, and DuBois, made confirmatory observations on the human subject (Lusk (*h*)). One hundred grams of glucose causes an average increase of nine per cent in the heat production of a man of 75 kilos; and 200 grams one of 12.5 per cent during 3 to 6 hours after the ingestion. The same dose with a smaller man produces a proportionally greater acceleration of the metabolism. Lusk and his pupils have found that the period of highest metabolism after heavy sugar feeding to dogs coincides with an

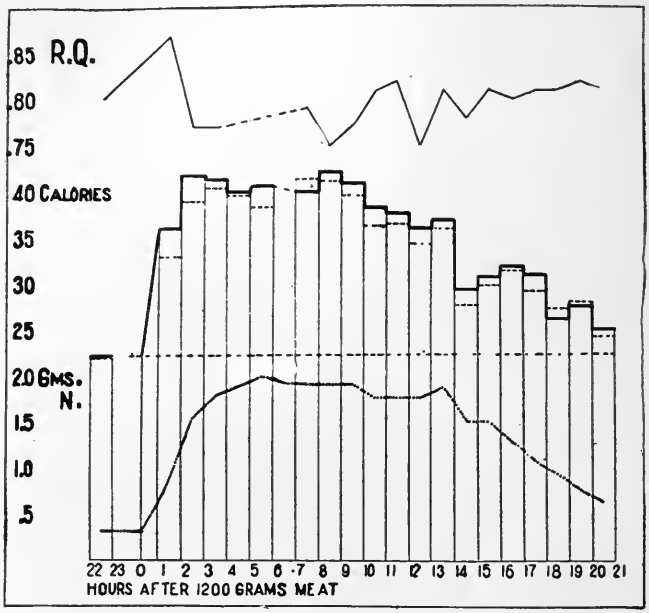


Fig. 34. After Williams, Riche and Lusk, showing the R.Q., the total metabolism determined by indirect (heavy black line) and direct (broken line) calorimetry as well as the nitrogen elimination (dotted line) during hourly periods after the ingestion of 1200 grams of meat, by a dog.

osmotic dilution of the blood caused by the rapid absorption of the sugar, and a sudden fall in the metabolism coincides with a removal of sugar from the circulation by the liver and a rapid elimination of water through the kidney. Lusk believes, therefore, that the heightened metabolism following rapid absorption of fat or carbohydrate may be called a "metabolism of plethora," or, in words of one syllable, oil on the fire. Since a summation effect is produced when carbohydrate and an amino-acid or both are added at a time when fat is producing a maximal effect and from other considerations which need not be entered into here, Lusk infers that separate mechanisms for oxidation of several foodstuffs exist within the body cells.

V. Basal Metabolism

By way of summary of the preceding sections one may say that the three factors which have most to do with determining the level of the energy metabolism in the normal subject are muscular activity, external temperature and food. A subject removed from the influence of these three factors would be (a) completely resting; (b) at a comfortable temperature; (c) and would be observed several hours after the ingestion of food. The metabolism under these conditions would correspond to the minimal functional activity of the body and for this reason has been called basal metabolism after Magnus-Levy(*h*) (Grundumsatz). The term "maintenance metabolism" (Erhaltungsumsatz) has also been given by Loewy(*a*), and the term "standard metabolism" is preferred by Krogh(*c*) who points out that even under complete suppression of muscular activity the metabolism of the heart may amount to as much as 4 to 15 per cent of the total metabolism of the body, and the metabolism of respiration to a like amount. The true basal metabolism according to Krogh would be found by deduction of those quotas assignable to the heart muscle and the muscles of respiration.

Whichever term is applied it should be understood that this minimal metabolism is the line of reference for the measurement of the various functional increases such as that due to food or to muscular work. The term basal metabolism will be employed in this chapter as being considered more appropriate than either of the other terms suggested. It is useless in the writer's opinion to use as the reference line a minimal metabolism lower than that which is attainable in the normal subject. It is, however, a fair question whether the metabolism of sleep should be taken as the basal metabolism in man, or, whether the condition defined by Benedict and his co-workers as the post-absorptive condition combined with complete muscular rest gives the better line of reference. F. G. Benedict has shown that in a fast of 31 days the metabolism during deep sleep may be as much 13.2 per cent lower than the metabolism of the same subject while awake but lying perfectly still. In this series the increased metabolism could not be attributed to muscular activity for a comparison of the graphic records showed that the degree of muscular repose was even more nearly perfect in the morning experiments while waking than in the night experiments during which the subject slept in the bed calorimeter. There was also no question of influence of food in the alimentary tract; for during the entire period of 31 days the subject ate absolutely no food and drank only about 900 c.c. of distilled water daily. It is fairly certain, therefore, that the only cause of difference was that state of the nervous system which we recognize as sleep. Presumably the lower metabolism in this state is due to the more complete suppression of muscular activity owing

to the absence of reflexes, with possibly a factor due to the suppression of neural activity in the brain, spinal cord and peripheral nerves. In time it may become necessary to revise the standard conditions for basal metabolism and to include, in addition to complete muscular rest and complete alimentary quiescence, neural rest. For the present sufficient data do not exist to warrant the change in standard; hence, the basal metabolism as ordinarily defined will be used in this chapter to determine the influence of age, sex, physical characteristics, etc., in the normal individual.

Even under the most uniform conditions thus far applied the basal metabolism has been found to vary from day to day and from hour to hour in the same individual, and even more in different individuals. For example, Johansson found on himself an average CO_2 production per hour of 22.2 grams with an average deviation from the mean of 3.6 per cent. Nevertheless, he found this metabolism to remain constant within the variation given over a period of seven months. Magnus-Levy(*b*) observed a similar degree of constancy over a period of two years. In a series of 51 observations made during complete muscular rest upon an athlete Benedict and Cathcart found a standard deviation from the mean of 4.9 per cent. When different individuals are considered the variation is much greater. The simple average percentage deviation from the mean in 35 different subjects observed by Benedict was 13.9 per cent.

1. The Influence of Physical Characteristics.—From an exhaustive biometric study of basal metabolism in the normal human adult including 137 men and 103 women, Harris and Benedict find that the most intimate correlations are obtained when correction for body size is made by expressing heat production in calories per square meter of body surface.[7]

As regards the effect of body weight upon the energy metabolism Harris and Benedict find that an increase of 1 kgm. of weight in the adult man increases the consumption of oxygen on the average 2.27 c.c. per minute and the carbon dioxid 1.87 c.c. per minute; for women the values are 1.17 c.c. oxygen, and 1.02 c.c. carbon dioxid. A kilogram of body weight added to the adult increases the total heat production for twenty-four hours on the average 15.8 Cal. for men and 8.27 Cal. for women. There is also a distinct and independent correlation between stature and energy metabolism, but this is not so close as with body weight. For each 1 cm. increase in stature the heat production increases about 16.6 Cal. per day in man and 6.9 Cal. per day in women. The same authors find that there is no very high degree of correlation between heat production and heart activity as measured by pulse rate, unless correction is made for body weight or body surface.

[7] This admission the authors are obliged to make although they do not believe that the closer agreement between heat production by different individuals and their surfaces than between heat production and body weight is due to any causal relationship (see page 597).

Referred to body weight the metabolism even in men of nearly the same size and weight may differ considerably. The results obtained by Jaquet and by Caspari vary from 0.8 Cal. per kgm. and hour to 1.6 Cal. per kgm. and hour. The latter figure was obtained by Caspari upon a trained athlete. Benedict and Smith have also shown that athletes have in general a higher basal metabolism than untrained individuals of the same physical measurements. Fat persons generally have, as would be expected, a lower metabolism per unit of weight than lean ones; for the fat tissues are relatively inactive. Other differences on the basis of weight may be accounted for, to some extent at least, by differences in muscular tonus, and differences in "endocrine efficiency."

As a convenient reference point the average obtained by Tigerstedt from a long series of determinations of the basal metabolism in man (namely, 1.04 calories per kgm. and hour) should be borne in mind. The average individual variation from this average is roughly plus or minus 10 per cent.

The physical characteristic which has proved to be most useful as a criterion or measure of metabolism is the surface area of the body. Rubner's original study on full-grown dogs is given in Table 19. Here it

TABLE 19

INFLUENCE OF BODY SIZE ON METABOLISM (Rubner)

Weight, Kgm.	Body Surface in Sq. Cm.	Cal. per Kgm. and 24 Hrs.	Cal. per Sq. M. (Meeh) and 24 Hrs.
31.20	10750	35.68	1036
24.00	8805	40.91	1112
19.80	7500	45.87	1207
18.20	7662	46.20	1097
9.61	5286	65.16	1183
6.50	3724	66.07	1153
3.19	2423	88.07	1212

was demonstrated how much more nearly proportional to surface the metabolism is than to body weight. While it is true that absolutely basal conditions were not present the animals were not observed to move about to any considerable extent. The original observations of Richet upon rabbits likewise are worthy of repetition here. The heat given off by radiation from the animal's body caused the air enclosed within the walls of the calorimeter to expand and to displace water in the siphon (page 582). Heat is expressed in Table 20 as the number of c.c. of water displaced. The number expressing the surface of the animal was found by Richet by regarding the body as a geometric sphere. Since its weight (volume) is equal to $\frac{4\pi R^3}{2}$ and the surface by $4\pi R^2$, the volume would be to the surface as $4.2R^3 : 12.6R^2$. Finding R from the known weight (volume) the relative surface was obtained by multiplying the square of this number

TABLE 20

RELATION OF HEAT RADIATION TO SURFACE OF THE ANIMAL BODY (Richet)

Weight, Gm.	Surface (A Relative Number)	Heat Radiated Expressed as c.c. of Water Displaced	Heat Radiation per Unit of Surface
2100	786	119	129
2300	841	110	130
2500	889	115	129
2700	932	119	127
2900	976	125	128
3100	1021	130	127

by 12.6. It is evident, Richet concludes, that the production of heat is a function of the surface and not of the weight of the animal. More nearly basal conditions were observed in experiments accomplished later by Slowtzoff(*a*) on dogs and by Kettner on guinea pigs. The former calculated the surface by Hecker's formula ($S = 12.33 \times W^{2/3}$) and found that the oxygen absorption per unit of surface in animals of different size (5.04 to 38.9 kgm.) "remains fairly constant" ($\pm$ 10 per cent mean deviation from the average, as against $\pm$ 12.5 per cent on the basis of weight). Kettner found that the CO_2 production per 100 gm. body weight and hour varied from 0.108 gm. in the largest (full-grown) animals to 0.254 gm. in the smallest (and youngest), a difference of 135 per cent, while on the basis of surface the extreme variation was only 30 per cent.

In the human subject the comparison of basal metabolism per unit of weight with the basal per unit of surface is even more striking. The following table from Gephart and DuBois(*b*) shows how much more the metabolism of different classes of human individuals differs from the average for adult men on the basis of weight than on the basis of surface.

TABLE 21

COMPARISON OF BASAL METABOLISM PER KGM. AND PER SQUARE METER OF SURFACE (Gephart and DuBois)

Investigator	Subjects	a. Cal per Kgm. and Hr.	b. Cal. per Sq. M. (Meeh) and Hr.	Per Cent Variation from Average for Men	
				a	b
Benedict and Collaborators	79 men	1.08	34.7		
Lusk and McCrudden	Dwarf wt. 23 kgm.	1.21	32.3	12	—7
Murlin and Hoobler.	6 infants	2.69	36.3	150	5
Benedict and Talbot.	Average 10 normal infants under 1 month	1.95	25.6	81	—26
Benedict and Talbot.	Average 11 normal infants between 1 & 10 mos.	2.21	35.5	105	2

This table was prepared before it was appreciated how much the metabolism varies with age and before the new method of measuring surface area devised by DuBois and DuBois was completed, but it shows how even on the old basis the metabolism was proportional to body surface rather than to weight. DuBois and DuBois in reviewing the literature of surface measurement found that a consistent plus error occurs in the use of the Meeh formula which may rise in very fat individuals to as much as 36 per cent. By their own method (see page 596) checked with actual linear measurements they found a total error in the case of five individuals of widely different shapes of only 1.7 per cent. On the basis of the new method for surface area Gephart and DuBois(*b*) later gave the average basal metabolism of nine normal men whose surface had been accurately measured as 39.7 Cal. per square meter per hour. The extremes of variation in this series were + 4 per cent and — 6 per cent. Selecting fat and thin subjects from the work of Benedict, Emmes, Roth and Smith and that of Means the authors find that the fat and thin groups show a difference in metabolism on the basis of weight of 41 per cent while on the basis of "linear formula" (p. 596) for surface area the difference was only 3 per cent. The law of surface therefore must be held to apply to fat and thin subjects as well as to the so-called normal. Nevertheless a variation of plus or minus 10 per cent must be expected even in perfectly normal subjects; for there are variations in muscular tonus, in the specific activity of the endocrine organs and in the conducting properties of the skin as well as in other factors not so definitely predictable which must always preclude the establishment of a fixed and rigid standard. Means found for example an average for sixteen normal subjects of 38.8 Cal. per sq. M. by the DuBois linear formula and that all came well within the 10 per cent (deviation from average) zone. Harris and Benedict feeling that they had totally discredited the law of surface as a measure of metabolism turned their attention to the prediction of the normal basal metabolism by means of biometric formulas based on stature, body weight, age, and sex and claimed that by this means "results as good as or better than those obtainable from the constant of basal metabolism per square meter of body surface can be obtained by biometric formulas involving no assumption concerning the derivation of surface area, but based on direct physical measurements."

Boothby and Sandiford have tabulated 404 determinations of the "basal metabolic rate," as they call it, expressed in percentages above and below normal, using both the standard of DuBois and that of Harris and Benedict. The average rates obtained by the biometric formula of Harris and Benedict are 6.5 points higher than those obtained by the DuBois method. The same authors report that they have made more than 10,000 determinations of basal metabolism on healthy people and on patients suffering from disease and that "only occasionally have we found patients

who had metabolic rates beyond the normal limits established by DuBois which could not be accounted for by the presence of a definite pathologic condition."

This truly phenomenal uniformity of heat production, quite equal to the uniformity of body temperature in normal subjects, has been explained in various ways. Rubner following Bergman and Regnault and Reiset attempted to bring the heat production into causal relationship with heat loss as we have seen (p. 599). This attempted explanation has not been wholly satisfactory for the reason that, as Lefèvre has shown, physiological adjustments can be made by the skin which greatly modify the application of Newton's law of cooling. Rubner himself, therefore, is obliged to postulate "similar physiological conditions" (page 599) and to assume that the minimal metabolism (basal) cannot undergo rapid changes but is adapted to the usual conditions regarding loss of heat which the animal has to meet. V. Hoesslin(*b*) has subjected the hypothesis of Rubner to a severe test by keeping two exactly similar young dogs for a long time under widely different temperatures and determining their resting metabolism at the end. The rate of heat loss must have been continuously very different for the coats of hair at the beginning were the same. Later it became thicker on one dog and thinner on the other in very obvious response to the conditions of heat loss to which they were subjected. *But the basal metabolism was not altered.*

V. Hoesslin himself considers that the metabolism of a tissue depends upon the supply of oxygen, that the circulation (and consequently the oxygen supply) must for anatomical reasons be proportional to the two-thirds power of the weight (i. e., to surface) and that the correlation of energy exchange with surface finds its explanation in these purely mechanical conditions. Dreyer, Ray and Walker have given some plausibility to this view by the discovery that in both mammals and birds the blood volume, the sectional area of the aorta and of the trachea in animals of different size are proportional to the two-thirds power of the weight. The trend of this view is wholly away from the teleological view outlined at p. 602 in connection with the subject of heat loss, and probably more correctly reflects the attitude of the modern mechanistic physiology.

Dreyer has more recently attempted the application of a more general formula to the normal basal metabolism and has compared the results found with those obtained by the more elaborate prediction formula of Harris and Benedict. His formula is $K = \frac{W^n}{C \times A^{0.1333}}$ where W is the weight, n approximately 0.5, C is calories of basal metabolism, and A the age in years. Table 22 shows that he gets a somewhat more concordant result than is obtainable with the prediction formula.

2. Influence of Age on Basal Metabolism.—DuBois(*a*) first assembled the data for the influence of time of life from birth to old age upon the

TABLE 22

Authors	No. of Persons	Description	Average K $\frac{W^{0.5}}{C \times A^{0.1333}}$	% Av. Deviation from K	% Av. Deviation by H and B. Prediction form
Palmer, Means and Gamble	8	men	0.1037	3.7	4.4
Carpenter, Emmes, Hendry and Roth	31	"	0.1014	5.94	5.30
Magnus-Levy and Falk	10	"	0.1000	5.06	5.27
" "	5	old men	0.1045	9.90	10.36
" "	15	boys	0.1007	3.46	15.60
Gephart and DuBois	5	men	0.0989	6.10	7.37
DuBois and Aub ..	6	old men	0.0993	8.20	19.38
" "	8	Boy Scouts	0.0928	9.49	19.70

total heat production. His chart in terms of calories per hour per square meter of body surface appears below. In considering the causes of the altered rate of heat production, one must bear in mind first the differences in body form which themselves affect the relationship of body surface to body weight; secondly, the specific influence of different organs which not

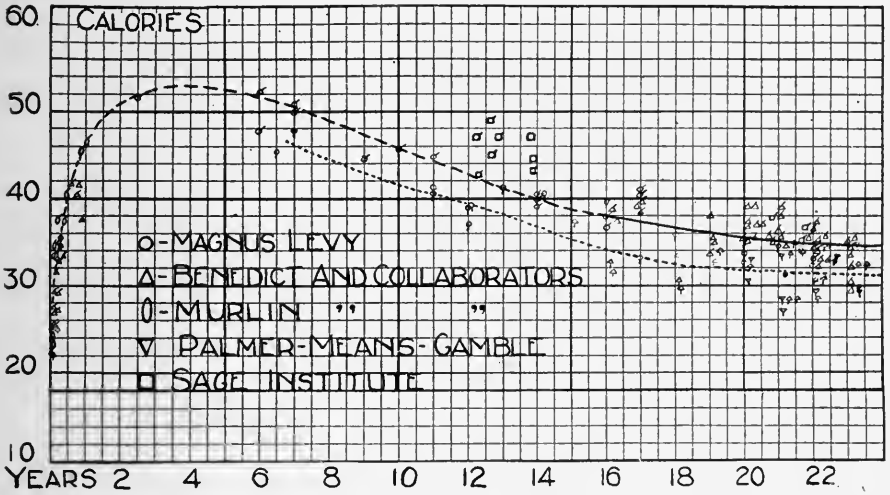


Fig. 35. Variations of basal metabolism with age: Calories per hour per square meter of body surface—Mech's formula. Dash line shows average for males, dotted line for females. After DuBois.

only bear different relations as regards size, to the body as a whole, but probably in some instances also have quite a different coefficient of activity. Thus, in early life the liver and thyroid, especially, both organs of high metabolic activity, are perceptibly larger in the relative sense than in the adult life, and may be expected to play a larger part in the total chemical activity of the body. This may, to a large degree, account for the

heightened metabolism of the infant one year old when reckoned on the basis of a unit of surface (Murlin and Hoobler). That the rate of growth itself, however, may be partly responsible, is evidenced by the fact that boys at the age of prepubescence, just when growth is accelerated, experience also a quickening of heat production. DuBois's results indicate that this may amount to as much as 25 per cent over the normal level for adults. Whether the awakened activity of the internal secretory mechanism of the sex glands acts independently or only through its effect upon growth, can only be decided by experiments upon animals. The latest experiments of this kind by Murlin and Bailey support the view of Loewy and Richter that in the female at least there is an independent effect quite outside the effect upon muscular rest. The tendency to obesity following the menopause in women is to be explained, therefore, as due to the absence of a stimulus which was present so long as the ovary was active. Removal of the ovary has the same effect. The falling metabolism of old age is to be explained in part by the tendency to reduce muscular effort of all sorts to a minimum, this, in turn, being traceable probably to the absence of internal stimuli, whether reflex or chemical. The deposit of calcareous material in certain organs, which so frequently accompanies old age, may also of itself reduce their metabolic activity.

Statistically studied, the decrease in total heat production per 24 hours for each year of age is, according to Harris and Benedict, 7.15 Cal. for their series of 136 adult men. For the 103 women it is 2.29 calories for each year of adult life. Upon the basis of a unit of body surface, the correlations with age "are of a more strongly negative character than the correlations between age and total heat production," which means that with each advancing year of life there is a heavier decline upon the basis of a square meter of body surface than upon the basis of total heat production. This conclusion is in accordance with DuBois's curve, though it does not give exactly the same rate of change.

3. The Influence of Sex.—Impressive also is the difference between the two sexes. DuBois had already drawn attention to this difference in the first curve which he published showing the variation with age. His curves for the two sexes ran about the same distance apart (7 per cent) as do the newer ones here reproduced. Twenty years ago Magnus-Levy and Falk found the difference between the two sexes both in early life and in advanced age about five per cent, but were of the opinion that in adult life the two sexes maintain about the same metabolism, consideration being had to difference in size and age. Harris and Benedict have analyzed the results of metabolism studies on the two sexes very exhaustively, making correction for body weight, body surface, age, and stature, and find that on every basis the metabolism for the women is lower than that of men. Even when the theoretical heat production of the woman is calculated by inserting their actual physical measurements in equations based on the series of men (regard-

ing the woman, that is, as a man of the same size) the actual heat production is generally lower than the theoretical. Larger women show a relatively larger deficiency than smaller ones and the suggestion is made by the authors that the body weight is the primary factor in determining the deficiency. "The most critical test shows that when body weight, stature, and age are taken into account, women show about 6.2 per cent lower metabolism than men."

D. Energy Metabolism of Growth

1. Differences between Growth and Maintenance.—The chemical processes by which the living substance is maintained are not identical with those by which it was originally produced. For example, growth and division of the nuclei are essential in the production of new tissues, while the mere replenishment of cell materials, such as is taking place continually on a small scale or such as may take place in convalescence on a large scale, may go on without division of the nuclei. Since it is known that the nucleus is essential to processes of intracellular digestion (Verworn), it is possible that the nucleus plays some essential rôle in this process of replenishment; but the fact that the nucleus itself does not grow and divide under these circumstances (Loeb, J.(*b*)), together with the fact that its reactions and constitution are known to be different from those of the cytoplasm, makes it very probable that growth involves chemical processes not concerned in the replenishment which follows ordinary waste or that which follows extraordinary waste in diseased conditions. Rubner(*cc*) has drawn attention to the fact that the maintenance tendency is of primary importance even in the young organism, since the "wear and tear" quota (Abnutzungsquote) must be satisfied before growth (postembryonic) of the organism as a whole can assert itself. If we assume that the everyday repair concerns mainly the cytoplasm, except where cells are actually being destroyed, Rubner's view might be interpreted to mean that the processes in the nucleus which result in its growth and division can take place, even in the young organism, only under certain optimum nutritive conditions of the cytoplasm.

There is no reason for thinking that the mechanism by which energy is liberated in young cells is different from that which performs the same service in mature cells. The living substance of all cells (with the exception of the anaërobic forms) is dependent upon some power, call it the "activation of oxygen," whereby oxygen becomes capable of uniting with the elements of the soluble foodstuffs at a temperature much below the ordinary kindling temperature.

Warburg's(*a*) recent observation that fertilized sea urchin eggs absorb six to seven times as much oxygen in the same length of time as do un-

fertilized eggs, lends weight to the view that oxygen is in some way essential to the growth process, but his further observation that there was no proportion between the amount of oxygen absorbed and the number of nuclei (blastomeres) present, and that still more oxygen was absorbed when the eggs were placed in hypertonic solutions and cell divisions had ceased (Warburg(*b*)), certainly do not favor the idea that oxygen absorption is dependent upon nuclear activity. This is in accordance with Rubner's(*m*) view that the morphological changes in the nucleus accompanying cell division are the expression of synthetic processes rather than of the destructive processes of oxidation.

Bayliss(*b*) explains the chemical process of oxidation in the cell as follows: "Some autoxidizable substance in the cell takes up molecular oxygen, with the formation of peroxids and activation of half the oxygen. The other half of the oxygen serves for *complete* oxidation of part of the autoxidizable substance. These peroxids are acted upon by peroxidase, with further increase of active oxygen, which is able to bring about oxidation of substances not autoxidizable and otherwise difficult of oxidation." The *structure* of the cell, however, also plays a part. For example, according to Warburg(*c*), in a muscle cell a much larger part of the chemical energy appears as free energy than if the cell is disintegrated. The arrangements within the cell which we call cell structure "in some way catch the chemical energy of the oxidation processes before it has fallen to the state of free heat." It is by such arrangements or structure that the work of a contracting, a secreting, an absorbing cell, etc., is carried on.

Even in cells which do no external work or osmotic work, however, structure is important for oxidation. Thus, in the unfertilized eggs of the sea urchin, Warburg and Meyerhof have shown that the addition of iron salts increases oxidation very perceptibly. Salts of no other metal do this. Iron, in other words, is a catalyst for oxidation. Now the significance of structure (alveolar, if we please), as Warburg sees it, is just this, that it affords surfaces for the condensation of the catalyst and thereby puts it to work.

But why should energy be set free in cells that do no work? Warburg's answer to this is that the liberation of energy by oxidation preserves the structure, or the integrity, if one will, of the living substance. If cell constituents are to be prevented from mixing freely, diffusion surfaces must be maintained, and the maintenance of their semi-permeable properties calls for a certain difference of electric charges which can only be kept up by the liberation of energy from some source. Hence it is that all living substance must respire and must liberate a certain amount of free heat. The maintenance of a constant temperature would, on this view of the matter, be a fundamental property for cells whose *structure* could be maintained only by a certain rate of energy release (see page 602).

2. Metabolism of Embryonic Growth (Murlin(*c*)).—Development oc-

casions a more active production of carbon dioxid per unit of mass than takes place in adult tissues. This has been demonstrated by Farkas for the eggs of the silkworm, by Bohr for the embryo snake, by Bohr and Hasselbalch, and by Hasselbalch alone for the developing chick, and by Bohr for the embryo guinea pig. That this greater evolution of carbon dioxid is the expression of a greater liberation of energy also is rendered perfectly certain by the calorimetric measurements made by Farkas of the heat of combustion of unincubated and incubated silkworm eggs and those of Tangl on the eggs of the cadaver fly; by similar measurements made by Tangl and by Tangl and Mituch on unincubated and incubated hen's eggs; and by the direct calorimetric determinations of the heat produced in the developing hen's egg made by Bohr and Hasselbalch.

Bohr and Hasselbalch found on the fifth day of incubation of the hen's egg a production of CO_2 amounting to 2000 c.c. per kilogram of embryo per hour as against 718 c.c. per kilogram and hour for the adult hen (Regnault and Reiset). The CO_2 production from the eighth to the twenty-first day (end) of incubation was only a little greater in the embryo than in the adult hen, but was sufficiently high for the authors to feel justified in concluding that it was "a condition for the organization of the new tissue and not alone for the maintenance of tissues already formed." Grafe, in reviewing this work, lays special emphasis on the fact that the highest energy production takes place at a time when the work of differentiation is most active. Bohr had previously supported this view with the evidence derived from his study of embryo snakes. Increasing the temperature from 15° C. to 27° C. increased the CO_2 output of an embryo weighing 3.8 gm. about 2.8 times, while the same increase in temperature raised the output of an embryo weighing 0.5 gm. exactly four times. The greater increase produced in the younger embryo, Bohr believes, was due to the greater change in the intensity of the developmental processes. That is, the processes of new formation (differentiation) are more active in the younger stage and it is this part of the developmental process which is responsible for the more active metabolism.

Tangl's results on the hen's egg indicate an average heat production for the entire incubation period of 100 calories per kilogram per day as against 71 calories per kilogram per day (at 18°-20°) for the adult hen found by E. Voit—an increase of 41.3 per cent. Tangl concludes that the energy required for development (Entwickelungsarbeit) is considerably greater than that required for mere maintenance of the adult organism (Erhaltungsarbeit). The difference he designates as Bildungsarbeit. Bohr's findings on the pregnant guinea pig are not so convincing. The average production of CO_2 in the embryo he found to be 509 c.c. per kilogram and hour; that of the mother 462 c.c. per kilogram and hour—an increase of only 10 per cent. Granted that the conditions of heat loss were the same in the two, which is doubtful, the amount of metabolism

which could be ascribed to any developmental process as opposed to the maintenance processes would be very small.

Rubner(*m*) believes that the law of skin area is applicable to the embryo. He calculated that the metabolism of the new-born mammal, assuming its weight to be 8 per cent of that of its mother, would be nearly double as much per kilogram and hour as that of the mother.

Because the embryo is less active in every way than the new-born its metabolism per unit of weight should be considerably less than this, which indeed the results of Bohr and Tangl show to be the case. Rubner explains the higher metabolism of the embryo per unit of weight, therefore, as due not to any specific requirement for developmental energy, but entirely to the greater loss of heat by the relatively greater surface. He is obliged, however, to eliminate the first four-tenths of the embryonic life from the operation of this law, because within that period the embryo is of no appreciable size as compared with the mother. On the basis of the average composition of living substance in mammals and using seven tenths of the metabolism of the new-born as the rate for the embryo, Rubner calculates that for the remaining six-tenths of the gestation period the "growth quota" of the embryo in most mammals is from 38 to 40 per cent of the energy supplied, as compared with 34 per cent for extra-uterine life. In other words, for each calorie of heat value stored in the new-born nearly two calories of energy must be expended, while for each calorie deposited in the embryo only one and one-half calories need be expended (on the basis of 40 per cent). We shall see that the higher metabolism of the embryo and fetus is continuous with that of the new-born.

The qualitative differences in the metabolism of the embryo from that of the adult depend on the kind of food material supplied by the mother in the egg (oviparous development) or by the circulation (viviparous) for the nutrition of the embryo. A hen's egg contains no carbohydrate; hence the respiratory quotient in development of the chick can never be greater than 0.78 (see page 560). The chemical studies of Liebermann, the calorimetric determinations of the heat of combustion by Tangl and the metabolism studies (using the direct and indirect methods) by Bohr and Hasselbalch all agree in showing that the material oxidized in the development of the chick is fat. Liebermann believed that some nitrogen was lost, but both Hasselbalch and Tangl and Mituch have shown that this is incorrect. The nitrogenous building material is not used as a source of energy.

The source of energy for the silkworm embryo, according to the chemical studies of Tichomiroff and the respiration experiments of Farkas; for the blow-fly embryo according to the respiration experiments of Weinland; and for the cadaver fly according to the calorimetric determination of Tangl is likewise mainly fat. No nitrogen is lost in gaseous form dur-

ing the development of any of these insects, but a portion of the energy (according to Farkas approximately one-third) arises from the oxidation of proteins to uric acid. Both Tichomiroff for the silkworm egg and Weinland for the blow-fly recorded a reduction of the glycogen content of the egg, but Weinland believes this may have been converted to chitin. There is no evidence, he says, that glycogen has served as a source of energy.

Our information as to what material is the source of energy for the mammalian embryo is extremely scanty. Cohnstein and Zuntz analyzed the blood in the umbilical artery and vein of the sheep embryo for oxygen and carbon dioxid, and noted a difference of 4.67 vols. per cent O_2 and 4.72 vols. per cent CO_2 in one case and 4.0 vols. per cent O_2 and 6.5 vols. per cent CO_2 in another. These figures would give respiratory quotients of 1.01 and 1.6 respectively for the two embryos. It is doubtful whether these figures are to be trusted, since on the basis of the same analyses the authors claim a metabolism for the embryo of only one-fourth to one-sixth as much per unit of weight as for the mother. The quotients agree, however, with those found by Bohr on the embryo of the guinea pig. Bohr took the difference between the total gaseous exchange of the pregnant animal (after operation under anesthesia and immersed in a warm bath) before and after clamping off a single umbilicus. The respiratory quotient indicated for the embryo was always in the neighborhood of unity. Oddi and Vicarelli report also a progressive increase in the course of pregnancy in the mouse. According to these observations, therefore, the most diffusible of the foodstuffs, the one most readily passed through the placenta is probably the source of energy for the mammalian embryo. There is no satisfactory evidence as yet that proteins participate to any considerable extent in furnishing such energy.

3. Metabolism of Post-embryonic Growth.—While metabolism is certainly more active in the youthful organism than in the adult it is by no means proved that the growth *per se* calls for any expenditure of energy. In recent times the view seems in fact to have gained rather general acceptance that the large metabolism of the young is necessary in the interest of heat regulation. At the same time the propensity to grow, which is the certain sign of youth in health, may be given a sort of energy index. There is a considerable body of evidence that growth in a given genus is proportional to the potential energy of the food consumed, and the proportion of gain in weight to energy intake may be quite similar in different genera.[8] It would seem that the growth impulse which, in some way not at all understood, directs and governs developmental events through the processes of nutrition, is geared, so to speak, at a very similar

[8] This statement, in view of recent developments in the realm of the chemically unknown accessory substances (vitamines), must be guarded by the saving proviso that an adequacy of these several substances is assumed.

speed in relation to energy intake in several genera and orders of mammals. A kilogram of body substance in several of them contains, according to Rubner(*cc*), 30 gm. N and 1722 calories of potential energy. To produce this unit of growth requires in the earliest period of postnatal development approximately the same amount of food energy; namely, 4088 calories. The human infant, however, occupies an exceptional position, in this regard, which may be expressed as follows. Of 100 calories of energy in the form of milk there is utilized for growth in the—

Colt	33.3%
Calf	33.1%
Lamb	38.2%
Pig	40.2%
Puppy dog	34.9%
Kitten	33.0%
Young rabbit	37.7%
Average	34.3%
Human Infant	5.2%

The average ingestion of milk in relation to the maintenance requirement (this term in Rubner's usage is not synonymous with basal metabolism) in the mammal is 202 per cent, while for the infant it is only 120 per cent.

The relatively long infancy period in the human family, it would seem, is a consequence rather than a cause of this difference; for if the large amount of time spent in sleep explained the low intake of food, and the slow development were a consequence of this, then keeping the baby awake and thereby increasing the demand for food ought to accelerate its growth. Of course just the opposite is true. Owing to a growth impulse of low speed, which in turn probably determines capacity for food (anatomical capacity of the stomach and functional capacity of metabolism) on the part of the infant, the human mother is called upon to supply intelligent care and protection rather than bulk of nutrients. Interesting biological implications are involved which space does not permit us to develop at this time.

It is doubtful whether the growth quota of energy, i. e., the portion left over after the maintenance factor, the activity factor, the dynamic factor and the loss by non-absorption have been covered, can ever be fixed as a definite percentage of the maintenance metabolism for all varieties of infants. The growth impulse, as between individuals, quite as truly as between different orders of animals, is more a matter of heredity than of food. Moreover, it is inherited from the father equally with the mother, so that a small mother nursing the child of a large father may not be able to supply milk enough for the rate of growth which the child has inherited. Again it is well known that growth in height often will proceed at a time when nutrition is not sufficient to support growth in weight, and both vary with the season of the year (Porter, Bleyer). In time we shall have in

addition to statistical criteria, physiological norms of growth which will simplify the whole problem of infant feeding. At present it is impossible to formulate even a satisfactory physiological definition of the growth rate. Merely to emphasize the multiplicity of factors contending for energy before growth can be wholly satisfied and to visualize what is known of their quantitative relations, the following tabular arrangement may be presented:

Basal metabolism	60	Cal. per	kgm.
Activity metabolism (12 to 40% of Basal)	7.2 to 24.0	"	"
Loss by feces (10 to 15% of Basal)	6.0 to 9.0	"	"
Dynamic action (10 to 20% of Basal)	6.0 to 12.0	"	"
Growth (10 to 20% of Basal)	6.0 to 12.0	"	"
Total	85.0 to 120.0	"	"

This estimate is liberal in all divisions of the caloric needs. Careful reckoning of the fate of the food energy cannot account for more than is here allowed except in such extreme restlessness as would place the case clearly in the pathological field.

This classification is not to be looked upon as anything fixed. The basal requirement increases steadily up to one year of age or later. The requirement for activity increases steadily in the absolute sense as the child spends more and more time awake, but it is not yet certain whether the increase is also relative to basal needs on the basis of weight or surface. Utilization is not known to change with age, the results with very young infants being often quite as favorable as with older ones. Dynamic action has not been sufficiently studied to say definitely whether it is greater or less as more and more food is ingested at a meal. There are indications that it is greater. Finally, the requirement for growth relative to weight increases certainly for the first three months and possibly up to six months, after which it becomes retarded. We have yet to learn whether the growth increment (in calories) advances more or less rapidly than the basal requirement. Van Pirquet, who has recently invented a system of computing food requirements, obviously based upon energy units (and merely disguised as "nems") estimates the growth quota at one-third the minimal or maintenance requirement. From the observations of Soxhlet on the calf it has been estimated that this animal can utilize over 40 per cent of the food energy for growth but an infant of 7 months was able at best to so dispose of only 13 per cent. Mere fattening should not of course be included in growth.

E. Energy Metabolism of Pregnancy

The energy metabolism of the fetus immediately before birth has been determined separately only by noting the difference in respiratory exchange of the mother produced by clamping off the umbilical cord (see page 619). This method, however, is open to serious objection and has

not given satisfactory results. In pregnancy the extra metabolism due to the product of conception includes the energy used by accessory structures as well as by the fetus itself. Nevertheless, it is worth while to estimate the difference particularly with a view to determine whether any material change in energy relations occurs at the moment of parturition.

With the dog Murlin(*c*) was able to show that the extra heat production of mother and offspring just before parturition was very nearly proportional to the weight of newborn pups delivered three days later. It was impossible to record the metabolism nearer to parturition than this on account of the restlessness of the dog. Quite fortunately it happened that the same dog gave two litters, one consisting of a single, the other of five pups. Comparing the total metabolism on the third day before parturition in the two pregnancies with that of the dog in sexual rest after lactation had been stopped, it was found that the extra energy metabolism at the culmination of pregnancy for the one pup was (551.3 — 505.3 =)46 calories or 164 calories per kilogram of the single newborn pup; and (763.8 — 505.3 =)258.5 calories or 165 calories per kilogram for the five new-born pups. In other words, the extra metabolism was very nearly proportional to the weight of the newborn.

46 Cal. : 258.5 Cal. : 280 gm. : 1560 gm.

It should be emphasized that the temperature of the cage was the same on the several days compared, that the mother dog was trained to lie perfectly still, and finally that the diet was exactly the same in weight and composition on all these days.

It is interesting to observe that the extra metabolism necessary to maintain the embryo (and all accessory structures of the mother's body) at a time when the pregnancy is at its highest phase is very nearly equal to the amount which the newborn of the same weight would theoretically produce (according to the law of skin surface), the first day after delivery, if exposed to ordinary room temperature and if resting (Murlin(*c*)).

If the law of skin surface is applicable to the embryo and the newborn, as Rubner believes it is, we may conclude that the metabolism of the uterus, mammæ, etc., would almost exactly compensate for the difference between the metabolism of the newborn at room temperature and the metabolism of the embryo at the temperature of the mother's body. In other words, the curve of total metabolism of mother and offspring would scarcely suffer any interruption at birth, if mother and offspring after birth could be kept sufficiently quiet for the demonstration. If this generalization should be true of the human mother and her offspring it would be a matter of considerable interest and importance.

To secure proper conditions for this inquiry, the problem was taken to the Nutrition Laboratory of the Carnegie Institution in Boston, where a bed calorimeter had been perfected large enough to contain mother and child (Carpenter and Murlin). Three subjects were studied. The metab-

olism of the pregnant woman was determined a number of times throughout the last two or three weeks, and similar determinations were made upon

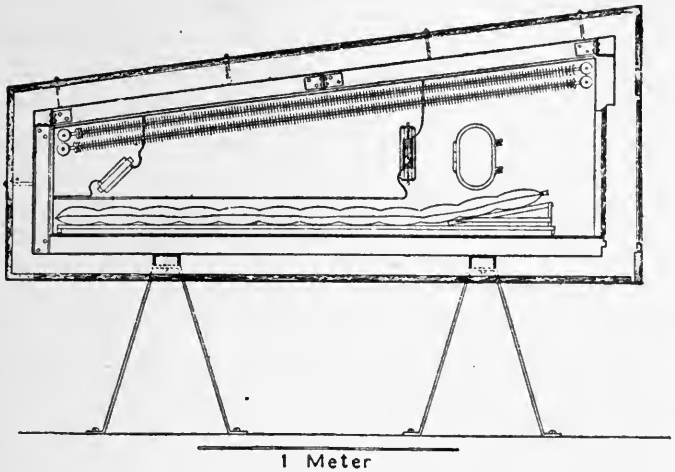


Fig. 36. Cross-section of bed calorimeter (Benedict and Carpenter), with which Studies on Pregnancy were made by Carpenter and Murlin.

the mother and child as well as upon the mother alone after parturition. A table showing the comparative results is given below.

TABLE 23

ENERGY METABOLISM OF MOTHER AND CHILD TOGETHER BEFORE AND AFTER PARTURITION (Carpenter and Murlin)

CASE Mean of All Days Before and After Delivery	Respiratory Exchange				Energy, Production, Calories per Hr.			
	Average Body Temp., °C.	CO_2 Gm. per Hour	O_2 Gm. per Hour	R. Q.	a—Direct	b—Indirect	$\frac{a+b}{2}$	% Difference
Case 1.								
1st, 4th, and 6th days before delivery	36.75	21.3	18.4	.85	60.0	61.3	60.7	.....
2nd, 5th, 12th, 14th, and 17th after delivery	36.9	20.2	18.5	.80	61.2	61.2	61.2	+ 0.87
Case 2.								
13th, 17th, 19th, 20th, and 22nd before delivery	36.68	22.3	19.6	.83	63.6	65.9	64.7	.....
2nd, 5th, and 11th after delivery	36.8	21.7	20.4	.78	71.1	67.5	69.3	+ 7.1
Case 3.								
1st, 3rd, 17th, 21st, and 24th before delivery	36.64	23.9	20.2	.86	72.2	68.7	70.6	.....
4th, 8th, and 11th after delivery	37.23	23.1	20.3	.81	70.8	68.6	69.7	— 0.9

The energy production expressed in absolute figures in both cases 1 and 3 is the same after as before parturition. In case 2 there was an increase of about 7 per cent in the postpartum over the antepartum metabolism. This can be accounted for by the fact that the child cried lustily at times on two out of three postpartum days and the crying disturbed the mother. One is justified, therefore, in the conclusion that the total metabolism of mother and child immediately after birth of the child is not greater in absolute amount than it was immediately before delivery. The extra metabolism of pregnancy, at its culmination, due in part to the activity of the accessory maternal structures as well as to the fetus, as in the dog, is just compensated by an extra metabolism set up in the new-born as it begins an independent existence. Since the mammalian embryo has no appreciable weight as compared with the mother until near the middle of the gestation period, it is easily understood why several workers (Magnus-Levy) using the Zuntz method failed to find any increase in the oxygen consumption per unit of weight in pregnant as contrasted with non-pregnant women; or if such an increase appeared at all, it became evident only comparatively late in the gestation period. This was confirmed with respect to the total energy production as computed from the output of nitrogen and carbon by the writer in a series of experiments on a pregnant dog. The only exception to the rule is a single case reported by Magnus-Levy in which he observed both an absolute and a relative increase in oxygen absorption as early as the third month of gestation.

Leo Zuntz(*b*) reported three cases on two of which he made observations by means of the Zuntz-Geppert method throughout the gestation period and on the third a few observations in the sixth month only. He compared the results with figures previously obtained from the same subject in sexual rest. The first two increased considerably in weight during the gestation period, quite independently of the product of conception, so that the amount of oxygen absorbed, when expressed per kilogram of body weight, was even less in the ninth month (Case C) than it had been in sexual rest, or was so little greater (Case B) that Zuntz believed the difference was entirely due to the increased labor of respiration. In the third case, however, the weight was less in the sixth month than it had been previous to conception, the oxygen absorption being as a consequence significantly larger per unit of weight in the pregnant condition. On the basis of this experiment and that of Magnus-Levy, Zuntz concluded that at the end of pregnancy the respiratory metabolism normally would be considerably higher than in sexual rest and that this is not altogether due to increased labor of respiration. Carpenter and Murlin compared their determinations on three normal cases of pregnancy with basal determinations on seven normal, non-pregnant women ranging in age from 18 to 55 years and in weight from 37 to 66 kilograms. Table 24 presents a comparison of the energy metabolism in the ninth month of pregnancy

TABLE 24

COMPARISON OF THE ENERGY METABOLISM IN PREGNANT AND NON-PREGNANT WOMEN, COMPILED FROM ALL SOURCES KNOWN TO-DAY

Subjects	Pregnant (Ninth Month)				Non-Pregnant				Remarks
	Weight, Kg.	O_2 Abs. c.c. per Kg. and Min.	R. Q.	Heat Production, Cal. per Kg. and Min.	Weight, Kg.	O_2 Abs. c.c. per Kg. and Min.	R. Q.	Heat Production, Cal. per Kg. and Min.	
Magnus-Levy's case (1896-97)	115	3.3	...	....	108	2.9	...	....	
L. Zuntz's case A (1905)	50	3.9	.79	....	50.8	3.47	.85	....	Sixth month of pregnancy
L. Zuntz's case B (1904-5)	58	3.7	.87	....	48.6	3.5	.84	....	
L. Zuntz's case C (1903-4)	67	3.4	.84	....	54.7	3.75	.81	....	Mean of all 4 cases to this point 3.4 c.c. O_2
C. and M. case 1 (1909)	63	3.4	.85	0.96	51.4	3.46	.85	1.06	1st, 4th, and 6th days prepartum; 15th day postpartum
C. and M. case 2 (1909)	58	3.9	.83	1.11	48.5	4.12	.78	1.23	Mean of 13, 17, 19, 20, and 22 days prepartum and 4th and 11th days postpartum
C. and M. case 3 (1909)	69.1	3.4	.85	1.02	60.1	3.34	.83	1.00	Mean of 1st, 3d, 17th, 21st, and 24th days prepartum; 9th and 12th days postpartum
Average of eight normal women, Carpenter (1910)	.....	Mean of 1, 2, and 3			....	3.65	....	1.10	
Average of all cases	.....	3.57	.84	1.03	37-66	3.48 3.49	.88 .88	0.99 1.02	

with the metabolism of the normal, non-pregnant woman, so far as the former has yet been studied.

It is surprising how close is the agreement between the results obtained with the respiration calorimeter and those obtained by the Zuntz-Geppert method. For example, Zuntz's case 3, agrees perfectly as far as the O_2 absorption is concerned with Carpenter and Murlin's cases 1 and 3. The mean oxygen absorption per kilogram and minute in the non-pregnant woman *before conception* is 3.45 c.c., for the eight normal women 3.48 c.c., but for the three cases taken during the puerperium it is 3.65 c.c., an increase of 5.8 per cent. The mean result for all non-pregnant women is 3.49 c.c. O_2 kilogram and minute. For the pregnant woman the result is 3.57 c.c. or 3.5 per cent more than the amount obtained for all the cases taken in complete sexual rest, and 2.2 per cent less than the average for the puerperium.

For the heat production Carpenter and Murlin found 1.03 Cal. per kgm. and hour for the pregnant cases as against 1.02 Cal. per kilogram and hour for all the non-pregnant subjects. For the woman in complete sexual rest, however, the mean result for the eight cases is 0.99 Cal. per kilogram and hour, i. e., about 4 per cent less than for the pregnant woman. The agreement between the oxygen difference and the total energy difference is very satisfactory. The conclusion which may be drawn with entire confidence is, that *the basal energy metabolism expressed per kilogram and hour, of the pregnant woman in the last month of her pregnancy, is but little larger* (4 per cent) *than for the woman in complete sexual rest.*

While we have but little data as to the depth of respiration or as to the increased labor of respiration in pregnancy, one may be inclined to think that so slight a difference might be attributable entirely to such a cause, instead of only partly so, as L. Zuntz believed. In fact, according to Zuntz's own estimate of the increased labor of respiration in his Case B the difference in oxygen absorption between the pregnant and the non-pregnant condition is exactly accounted for in this way. This conclusion would mean, very clearly, that the metabolism of the fetus, together with all accessory structures, is the same as so much maternal tissue. If the metabolism of the fetus itself were slightly higher in the human, as it seems, from Bohr's experiments, to be in the guinea pig, this factor would be counterbalanced by the fact that the liquor amnii (and possibly the membranes) takes no part in the metabolism.

On the other hand, the heat production in the puerperium is distinctly higher than that for complete sexual rest or for the pregnant condition—the average for Carpenter and Murlin's three cases being 1.10 calories per kilogram and hour, or 11 per cent higher than the average for the former and 7 per cent higher than the average for the latter.

What is the explanation of this higher energy production of the puerperient mother? That it was not fever is apparent from the very accurate

temperature measurements made by rectal thermometer. It is conceivable that the processes of involution, which were not yet entirely complete at the time of the above observations were made, set free decomposition products which stimulate the general heat production in a manner analogous to the stimulation of the mammary glands by fetal products. If so, the processes by which heat is lost from the body (evaporation of water, radiation and conduction) must be equally stimulated, for there is no accumulation of heat. A state of hyperactivity of the sweat-glands, especially during the early days of the puerperium, is a phenomenon well known to obstetricians and it is possible that this activity is a primary cause of the increased heat production—a cooling of the body surface generally resulting in a reflex stimulation of the heat-producing tissues. The writer believes, however, that the most important factors are the activity of the mammary glands and the specific dynamic action of the foodstuffs burning—especially the increased protein combustion due to involution of the uterus. The lower respiratory quotient found in the puerperium is to be ascribed to the restricted diet very commonly imposed immediately after delivery, and is a sign that the patient has used up her store of glycogen during labor and is thrown back on her reserve of fat, and on the protein resorbed from the uterus for her supply of energy. The dynamic action of the latter would considerably increase the heat production.

F. Energy Metabolism of the Newborn Infant

1. The Respiratory Quotient of the Newborn.—In the observations of Mensi, Scherer, and Babák, the respiratory quotient of the newborn child was found to be extremely low, so much so that it was inferred that oxygen must be utilized in the infant's body for some other purpose than that of combustion. More recent observations have discredited this interpretation, for it has been rendered very probable that the technique of the early observers was seriously at fault. Hasselbalch points out that Scherer's oxygen must have contained a much larger percentage of nitrogen than he assumed, from an old analysis, to be present; also that there was an admitted error of 6 per cent on the carbon dioxid.

Hasselbalch(*a*) himself obtained quotients which were much higher. Since his technique seems to have been carefully controlled, it is probable that his results are much more reliable. In fact, Hasselbalch lays stress on the fact that the R. Q. of the newborn infant before it begins to take food is often much higher than that of an adult in a similar state of inanition, and he thinks it is fair to infer that in such cases, which in his tables include both the well-nourished infants born at term and infants prema-

turely born, there is a plentiful amount of glycogen available at birth and it is the requisition upon this reserve carbohydrate which produces the high quotients.

Hasselbalch infers much from the single experiment of Bohr on the pregnant guinea pig (quoted at page 619) showing that the respiratory quotient of the embryo is 1.0. It is quite possible that this is true, but the single experiment of Bohr can hardly be accepted as proving the case beyond doubt. Recent analyses of the blood of the mother and of the umbilical vein taken simultaneously at parturition show clearly that other materials than glucose can pass the placenta very readily, and while one may be prepared to believe that the main reliance of the embryo for energy is the most diffusible of the foodstuffs, it must not be inferred that no other substance is available for combustion in the fetus. Were carbohydrate the only fuel available during antenatal life, it might be argued that the enzymes are not yet ready for liberation of energy from fat (which certainly is present), even if a large store of glycogen could not be demonstrated; and we might expect to find the quotients rather higher immediately after birth than a little later. Hasselbalch himself admits that the facts are not quite so easily explained. Referring to Table 25 it is seen that the highest quotients do not always come at the earliest hour. When the same subject was used in two successive experiments, however, this was found to be true.

So convinced was Hasselbalch that the quotient was higher the better the state of nutrition of the newborn that he thought he could tell when the quotient was lower than 0.9 by signs of hunger in the infant.

The occurrence of high quotients within the first seven or eight hours after birth was observed independently also by Bailey and Murlin. They drew attention to the particular interest which the quotient at this time presents, as indicating the kind of material available for combustion as the child breaks connection with the maternal circulation. They were on their guard, however, against inferring, without further information regarding the absorption of oxygen at this age, that the high quotient necessarily proves a predominantly carbohydrate combustion. "Assuming that oxygen absorption is normal at this age," they say, "the quotients obtained would indicate the combustion of a considerable amount of carbohydrate (glycogen)." Since Morris has published his sugar analyses in maternal and umbilical bloods and has shown that the level of the blood sugar is raised in both by a severe labor or by the use of an anesthetic, another explanation of the high quotients which are met with in the early hours of postnatal life has been presented. Henceforth it will be necessary to know something of the severity of labor and whether the mother was given an anesthetic, before a plentiful supply of glycogen in the liver of the newborn all ready for combustion the moment the cord is tied, can be inferred. However, it is possible that the severe labor would

TABLE 25

RESPIRATORY QUOTIENT OF THE NEWBORN INFANT (Hasselbach)

Experiment No.	Sex	Body Weight, Gm.	Height, Cm.	Age Hr.	Age Min.	Remarks on Food and Muscular Repose	CO_2 Elimination per Kg. per Hr. at 0° C. and 760 mm.	R. Q.
9	F	3,750	51	..	45	No food; quiet throughout; slept latter half of experiment	333 c.c.	0.970
3	M	3,100	51	1	30	No food; bath; crying and kicking 1 minute; otherwise contented; sucking or half asleep	481	.868
8	F	3,950	54	1	30	No food; quiet, now and then sucking; otherwise without movements throughout experiment. No crying	270	.862
12	F	4,000	54	1	30	No food; rather restless; hungry; now and then crying	399	.794
2	F	3,650	51	2	..	No food; during most of experiment quiet and contented; now and then sleeping; cried about ½ minute; fat and strong	422	1.012
7	M	3,200	50	2	..	No food; rather quiet; now and then kicking and trembling as if cold; no crying	457	.909

mobilize glycogen from the maternal tissues and that ether administered would mobilize it from both the maternal and fetal tissues, so that the umbilical vein would get a contribution from both directions. Hasselbalch's insistence upon a relationship to general nutritive condition is not necessarily discredited, for it is well-known that in the majority of instances a large, well-formed infant produces a more difficult labor which itself, without the assistance of an anesthetic, would in all probability call out enough carbohydrate into the circulation to raise the quotient several points. Premature infants also produce an easy labor, and this fact with absence of a hyperglycemia may explain the impression of Hasselbalch that in the prematurely born infant "the store of carbohydrate is very quickly spent."

Benedict and Talbot(*a*)(*b*) did not observe especially high quotients immediately after birth; for the technique of their experiments was not calculated to separate the respiratory quotients into individual periods. The authors state, however, that when the quotients above and below 0.80 are compared, it is found "that up to the eighth hour the greater number lie above 0.80, while subsequent to the tenth hour the larger proportion lie below this value."

All the modern observations agree in showing a rapid fall in the respiratory quotient toward the end of the first day. Hasselbalch did not repeat his observations on the same infant except in immediately succeeding periods; but even these second periods show in four out of five cases a noticeable fall. Bailey and Murlin made observations on two infants born three hours apart on the same day and placed in the respiration incubator at six hours of age. The observations were repeated on the second, fourth, fifth, and sixth days with one child, and on the second, fourth, fifth, and eighth with the other. The quotients fell to 0.67 in both cases on the second day. While distrusting the exact figures obtained, the authors point out the extreme significance of the indication, confirmed on a third newborn at the twenty-seventh hour, that all available carbohydrate has been utilized by this time, and the importance of supplying artificially, if need be, some materials to protect the body substances. Mother's milk was available in small amount for both infants on the third day, but the quotients did not reach the level usually obtained after breast feeding of older babies until the sixth day in one instance and the eighth in the other. These observations were confirmed by Benedict and Talbot in their long series, the values shown in Table 26 having been obtained as averages of several short periods for each infant.

a. *The Influence of Food on the Respiratory Quotient.*—Milk appears in the mother's breast usually by the fourth day, and by the fifth day the infant receives enough to prevent further loss in weight. The course of the average respiratory quotient from the first to the eighth days reflects the adequacy of the food intake. Unless artificial feeding

TABLE 26

RESPIRATORY QUOTIENTS THE FIRST EIGHT DAYS (Benedict and Talbot)

DAY	1	2	3	4	5	6	7	8
Respiratory Quotient	0.80	0.74	0.73	0.75	0.79	0.82	0.81	0.80
Number of Cases	74	64	62	51	41	22	15	9

is resorted to, the modern infant is doomed to almost complete starvation for the first three days, although it is clear, even from the average R. Q. in the observations made at Boston, that glycogen is present in sufficient quantity to prevent starvation acidosis the first day. When milk comes in sufficient quantity on the fourth day, the average respiratory quotient responds noticeably and on the fifth and sixth days mounts to a level which indicates a satisfactory state of nutrition.

The question has often arisen whether the newborn infant is capable at once of digesting and metabolizing a sufficient quantity of breast milk even if it were present, to prevent loss of weight. The answer to this question must be sought by means of the respiration apparatus. The matter will be discussed in its quantitative aspects at greater length beyond. Meantime, it may be noted that Hasselbalch has tested the capacity of the newborn to absorb and metabolize grape and milk sugar and that perfectly satisfactory evidence was obtained from the respiratory quotient that this capacity is developed by the end of the second day.

Infants born prematurely may have a high R. Q. within the first few hours after birth but by the fifteenth hour the supply of glycogen, or the hyperglycæmia due to labor or anesthesia or both, has been considerably reduced and the child may be already on a nearly pure fat metabolism. When an adult mammal already well nourished is given even a small quantity of an easily absorbed sugar, the effect upon the R. Q. may be seen within the first half hour. When, on the other hand, fat is given in large amount, the effect upon the quotient may not be seen until the third to sixth hour. We may expect then that in feeding an infant with milk, whether mother's or cow's milk, it is the sugar of milk which is burned first and the fat will only be absorbed in sufficient quantity to affect the R. Q. after several hours.

The work of Hasselbalch demonstrates these points very clearly. After feeding infants 2 and 4 days of age with breast milk, he found the highest quotient (.92 and .93) 1½ hours after the meal. In one case he was able to show that an experiment begun 2 hours after a feeding gave a quotient 4 points lower than an immediately succeeding period begun only one hour after a similar feeding. Apparently in Hasselbalch's experiments, as in those of Bailey and Murlin, it is much easier to secure this rise of quotient with infants five days or more of age than it is with those of 2 days or less. The explanation clearly is that unless artificial

nourishment has been resorted to, the infant's tissues are depleted of glycogen at 2 days just as are those of an adult after several days of fasting, and anything less than a large feeding of carbohydrate is held up by the tissues to satisfy their craving for storage glycogen.

2. **Basal Metabolism in the Newborn.**—Carpenter and Murlin found the metabolism of the newborn taken per unit of weight to be two and a half times that of the mother lying in bed beside the child. Later observations by Benedict and Talbot(*b*) and by Bailey and Murlin make the figure for newborns less than a week old 1.75 and 1.87 calories respectively per kilogram and hour as against 1.0 calory per kilogram and hour for the normal adult. The figure given by Benedict and Talbot is the average of observations on nearly one hundred subjects which ranged from two and a half hours to seven days of age, and had an average age of two days. That given by Bailey and Murlin is the average of twelve hourly periods on four infants less than one week of age, during which the infant slept all or substantially all of the time. On the basis of twenty-four hours at the same rate, the metabolism would be 42 calories per kilogram according to Benedict and Talbot, or 45 calories per kilogram and twenty-four according to Bailey and Murlin. It should be noted, however, that the periods selected for this average represented the periods of unusual muscular repose, and that no infant would ever actually maintain a metabolism so low for an entire twenty-four hour period. It avoids confusion, therefore, to report all results of metabolism experiments done in short periods on the hourly basis; for it is obvious that when a child sleeps quietly for the entire period, as it did in most instances in the two series of experiments referred to, the metabolism obtained does not represent an average condition for the entire twenty-four hours. In fact, it would be next to impossible to find a short period or to arrange conditions for one which could be said to represent average conditions for twenty-four hours. Moreover, a child does not metabolize materials in periods of twenty-four hours as an adult may be said on certain grounds to do. If there is any cycle of metabolism in the newborn, it corresponds to the feeding period.

The influence of weight on the metabolism per unit of weight is well illustrated by the table on page 633 from Bailey and Murlin. The metabolism is noticeably higher for a light-weight baby (W, birth-weight 6 lbs.) than for a heavy baby (B, birth-weight 10 lbs. 3 oz.). From considerations which will be presented in discussion of metabolism of older infants, it is practically certain that the principal factor responsible for such a difference is the insulating effect of subcutaneous fat or of the effect of fat to reduce the effective radiating surface.

The average heat production of all of the infants over 4.00 kilos body weight and over one day of age in Benedict and Talbot's(*b*) Table 12 (loc. cit. p. 95) is 1.75 calories per kilogram and hour, while the average

TABLE 27

	Weight, Kgm.	Age, Hours	Cal. per Hour	Cal. per Kgm. and Hour	Cal. per Sq. Meter and Hr. (Meeh)
W.	2.9	6	5.649	1.94	23.67
B.	4.6	6	6.724	1.46	20.43
W.	2.82	31	6.255	2.22	26.54
B.	4.49	31	9.704	1.94	26.87
W.	2.75	80	5.972	2.18	25.57
B.	4.27	80	7.101	1.66	22.67
W.	2.75	104	5.252	1.83	21.85
B.	4.27	104	7.500	1.77	23.47
W.	Average	...	5.782	2.04	24.43
B.	Average	...	7.514	1.70	23.36

of all those between 2.70 and 3.00 kilos in weight and within the same range of ages is 2.00 calories per kilogram and hour. The observations of Benedict and Talbot are thus in substantial agreement with those of Bailey and Murlin. One cannot say, however, that every individual case in these groups as compared with every other shows a metabolism which is inversely proportional to weight. The influence of body weight (fat) can be shown best by contrasting the extremes.

Within the age of one week the metabolism is by no means constant. The average of 31 cases less than 12 hours of age is, according to the results of Benedict and Talbot, 1.59 calories per kilogram and hour, while for their ten infants from 12 to 22 hours of age it is 1.87 calories. Beyond the first day there is but little fluctuation in the average. Thus for fourteen infants two days old the average is 1.86 calories per kilogram and hour and for thirteen infants four, four and a half, and five days of age, the average is 1.85 calories. It is evident from these calculations that the lower value noted above for Benedict and Talbot's longer series is due to the large number of infants less than 12 hours of age included in their observations. Summing up all the modern results, it may be stated categorically that the metabolism per unit of weight for the first twelve hours is approximately 15 per cent lower than it is the rest of the first week.

3. Metabolism of the Newborn Infant per Unit of Body Surface.—When the metabolism per unit of surface area of the newborn is compared with that of the adult, account must once more be taken of the actual age. The average for the first two weeks may be illustrated by the following table from Carpenter and Murlin slightly modified by Lusk(*b*). Here it is seen that the metabolism of the pregnant mother with an average weight for the three subjects of 63 kilograms was 33.4 calories per square meter of body surface (Meeh's formula). After parturition the average weight was 53 kilograms and the heat production 33.2 calories per square meter.

TABLE 28

METABOLISM BEFORE AND AFTER PARTURITION. THE METABOLISM OF THE CHILD WAS DETERMINED BY DIFFERENCE

	Weight in Kg.	Calories per Hour	Calories per Sq. M. (Meeh)	Calories per Kg. per Hour
Case I:				
Before parturition	63.0	60.7	31.4	0.96
After parturition	51.4	53.9	31.7	1.05
Difference	11.6	6.8		
Child	2.7	7.3	30.5	2.70
Case II:				
Before parturition	58.0	64.7	35.1	1.11
After parturition	48.5	59.0	36.2	1.21*
Difference	9.5	5.7		
Child	3.4	9.8	34.9	2.88
Case III:				
Before parturition	69.1	70.6	34.0	1.02
After parturition	60.1	60.4	31.9	1.00
Difference	9.0	10.2		
Child	3.2	9.3	34.8	2.90
Average:				
Before parturition	63.4	65.3	33.4	1.03
After parturition	53.3	57.8	33.2	1.09

* Child cried during experiments.

The average heat production for women between 20 and 50 years, according to Benedict and Emmes, is 32.3 calories per square meter. Now the still more remarkable fact is that the metabolism of the child (determined by difference between the metabolism of mother and child taken together and mother alone) with an average body weight of 3.10 kilos is 33.4 calories per square meter of body surface—exactly the same as that of the mother whether before or after parturition. A more striking agreement in accordance with the law of surface area would indeed be difficult to find. A woman heavy with child, the same woman immediately after delivery, the child itself, and normal non-pregnant women differing enormously in weight and showing a metabolism per unit of weight differing two and a half times have the same metabolism when this is reckoned on the basis of surface. The agreement, in fact, is too close to represent the exact truth, except for the circumstances presented by chance in these particular experiments. We now know from the further work of Murlin and Hoobler as well as that of Benedict and Talbot that the exact age makes a measurable difference in both the newborn and older infants. Nevertheless it holds as a substantial statement of the facts that the metabolism of the young infant (two weeks to two months of age) on the basis of surface area is the same as that of the adult. It is now known that the level of metabolism of the newborn less than one week of age is considerably lower than that of the adult. This discovery was made simultaneously by Benedict and Talbot, and Bailey and Murlin, though it was emphasized first

by the latter authors. According to Meeh's formula the basal heat production of the newborn was 23.7 calories per square meter per hour.

Benedict and Talbot interpret their results on all their infants between birth and one week of age as showing no relation between body surface and metabolism. Yet when two extreme groups like those mentioned on pages 632 and 633 are selected from their results, it is found that the average metabolism per unit of weight differs 12.5 per cent, while on the basis of surface area (Meeh's formula), the same groups show a difference of less than 3 per cent, namely 24.1 and 23.4 calories per square meter per hour.

The basal metabolism of the newborn above 12 hours of age while sleeping quietly at a comfortable temperature is in the neighborhood of 23 or 24 calories per square meter of surface, in contrast with that of the adult which is in the neighborhood of 32 or 33 calories. In other words, the metabolism of the newborn is nearly one-third less than that of the adult. On the same basis, the basal metabolism of the 31 newborn babies less than 12 hours of age in Benedict and Talbot's series is about 20 calories per square meter per hour or quite 40 per cent less than that of the adult. Singularly enough this same level of metabolism may be reached by the adult after twenty days of fasting.

4. Influence of Sex on Basal Metabolism of Infants.—From the sections immediately preceding, it is already evident that sex at this early age exercises little, if any, specific influence. Further examination confirms this impression. Thus the group of 31 infants under 12 hours of age in the Boston series includes 17 males and 14 females. The average weight of the males is 3.76 kilos and they have an average metabolism per kilo and hour of 1.53 calories. The average weight of the females is 3.29 kilos and they have an average metabolism per kilo and hour of 1.61 calories. The metabolism of the larger body is slightly less as before. The two groups, however, have exactly the same metabolism per unit of surface.

Carrying the comparison to older groups, we find the same is true of all infants two days of age. There are seven boys and seven girls of this age in the Boston series. The average metabolism of the boys is 1.85 calories per kilogram and hour, while that of the girls is 1.87 calories. The average metabolism per unit of surface (Meeh) is 23.5 calories for the boys and 23.2 calories for the girls. Using the DuBois height-weight formula and calculating the surface, the average for the boys is 30.7 calories and for the girls 30.4 calories. The mean percentage deviation from the average is slightly less for both groups on the basis of the Meeh formula than it is on the basis of weight or on the basis of the surface as estimated by the DuBois formula (Table 29).

Going on to infants 4 to 5 days of age, in the same series, we find the average weight of the boys is 3.34 kilos, that of the girls 3.83. The basal heat production per kilo and hour of the former is 1.88; that of the latter

TABLE 29

MINIMAL METABOLISM OF INFANTS 2 DAYS OF AGE (Benedict and Talbot)

MALES

No.	Wt. Kgm.	Ht. Cm.	Heat Prod. per 24 Hrs.	Heat Prod. per Kgm. and Hr.	Dev. from Mean %	Surface Area (Meeh)	Heat Prod. per Sq. M. and Hr.	Dev. from Mean %	Surf. Area, DuBois Formula	Heat Prod. per Sq. M. and Hr.	Dev. from Mean %
8	3.48	51.0	160	1.91	3.2	0.273	24.4	3.8	0.208	32.0	4.2
10	3.45	52.0	162	1.91	3.2	0.271	24.8	5.5	0.210	32.1	4.5
30	3.33	51.0	144	1.80	2.7	0.265	22.6	3.8	0.204	28.7	6.5
51	3.73	52.5	154	1.72	7.0	0.286	21.9	6.8	0.219	29.3	4.5
53	2.87	47.5	143	2.07	12.0	0.240	24.7	5.1	0.182	32.7	6.5
70	3.56	51.0	153	1.79	3.2	0.277	23.0	2.1	0.214	29.7	3.2
74	3.63	52.0	156	1.79	3.2	0.283	23.0	2.1	0.215	30.2	1.6
Average...	3.43	51.0	153	1.85	4.9	0.271	23.5	4.1	0.207	30.7	4.4

FEMALES

4	3.28	46.5	139	1.72	8.0	0.262	22.0	5.1	0.190	30.4	0.0
9	4.04	51.0	178	1.83	2.0	0.301	24.6	6.0	0.222	33.4	6.2
13	3.25	50.0	138	1.77	5.3	0.261	22.0	5.1	0.199	28.9	4.9
21	2.92	50.0	136	1.94	3.7	0.243	23.3	0.4	0.188	30.1	0.9
34	2.90	50.5	134	1.92	2.9	0.242	23.1	0.4	0.198	28.2	7.2
43	3.62	50.0	165	1.90	1.6	0.280	24.5	5.5	0.212	32.5	6.9
65	2.63	49.0	127	2.01	7.5	0.226	23.3	0.8	0.182	29.1	4.1
Average...	3.23	49.5	145	1.87	4.4	0.259	23.2	3.3	0.196	30.4	4.3

1.83 calories. On the basis of the Meeh formula the basal metabolism of the boys per square meter of surface is 23.5 calories and that of the girls 23.2. On the basis of the DuBois formula the metabolism is 30.5 and 31.0 calories per square meter per hour respectively. The mean deviation from the average is again less for the Meeh formula.

In the statistical analysis of the basal metabolism of the entire Boston series, Harris and Benedict carried the comparison somewhat further. They predicted the metabolism of girl infants from constants based on the boys, and determined the sign and magnitude of the difference between observed and calculated values. Equations employed were those showing regression of basal metabolism on stature (body length), on weight, and on body surface in the male infants. Subdividing the entire series of female infants into stature groups, it was found that out of six groups three showed a higher metabolism and three a lower metabolism than that predicted on the assumption that all were boys of like height. Classifying for surface area, out of seven groups four showed a higher metabolism and three a lower than predicted on the assumption that they were boys with the surface area of the girls. The comparison for body weight turned out the same. The authors conclude: "As far as our data go, they indicate that on the average there is no sensible difference between the heat production of the two sexes in the first week of life."

5. Influence of Crying.—Since the newborn child is scarcely able to influence metabolism by any other form of muscular effort than crying, the activity factor may be discussed under this heading. Bailey and Murlin cited among their results the case of a child ten days of age who produced 8.14 calories per hour while sleeping quietly throughout the period of observation. The next day, while crying "most of the time," i. e., one hour, she produced 10.73 calories, an increase of 31 per cent. Howland with Lusk's calorimeter observed an increase of 39 per cent in an infant 7 months of age for a one-hour period of "struggling and crying." Benedict and Talbot have contrasted in one of their tables minimal with maximal periods of activity (including crying) for 93 infants, and deduce an average difference of 65 per cent, the individual differences ranging from 4 to 211 per cent! Unfortunately 65 out of the 93 maximal periods are "calculated from the carbon dioxid produced during a preliminary period for which the respiratory quotient was not determined." Since even those periods for which oxygen as well as carbon dioxid was determined oftentimes gave "defective respiratory quotients due to excessive carbon dioxid excretion . . . or to a defect in the measurement of the oxygen, particularly the residual oxygen," it is impossible to compare Benedict and Talbot's results with those of Howland or Bailey and Murlin whose "crying" periods like their basal periods, were controlled by residual analyses. From a practical point of view, however, namely the effect of crying upon the energy requirement of the newborn, the several authors are in sub-

stantial agreement. For an infant who cries no more than the average normal infant probably 30 per cent increase above the basal would more than cover the energy requirement for maintenance; while for an infant who cries "most of the time" (admitting considerable latitude in the use of the expression), probably 40 per cent above the basal would be more than adequate; for it is certain that no newborn infant can continue to cry at a rate sufficient to increase the metabolism 40 per cent for more than a few hours out of the twenty-four.

6. Influence of Food and External Temperature.—Very few observations have been made indicating that the food of the newborn has any dynamic effect. Hasselbalch(*a*) reports two observations on premature infants in which he surmises that the increase of some 15 per cent in metabolism the second period is due to the "work of digestion." "At any rate," he asserts, "it was impossible to recognize a difference in the muscular activity of the infant." Since the first effect of hunger is to induce muscular activity in the form of crying, it is very difficult to secure complete muscular repose on empty stomach so as to have a basis of comparison with periods following the ingestion of food. In Hasselbalch's comparison just cited both periods follow the feeding and the more active work of digestion in the second period is inferred from the higher respiratory quotient. Coupled with the difficulty just mentioned is the natural reluctance of the physician to give the newborn a large feeding. In fact, it is quite possible that the stomach of the child at this time cannot contain enough food at a single filling to raise the metabolism sensibly.

We are equally without convincing evidence that external temperature acting independently can influence metabolism in the newborn. Scherer reported a difference of 23 per cent in oxygen absorption by the infant between what he called summer temperature (16 to 26.8° C.) and winter temperature (9.5 to 16.2° C.). But there was no control of muscular activity, or even notes regarding crying. Hasselbalch conducted his experiments at an average temperature of about 33° C.; Bailey and Murlin maintained a temperature of 27° to 29° C.; while Benedict and Talbot kept their chamber air at approximately 20° C. Hasselbalch is deeply impressed with the fact that his newborn infants (most of them only a few hours from birth) produced only 270 c.c. of carbon dioxid per kilogram and hour and that "this is not essentially higher than the corresponding figure for a grown individual in absolute repose." From the connection in which the author alludes to this comparison one might infer that the low metabolism which he mentions was due to the absence of all "chemical regulation" since the temperature was "so regulated that the question of the feeble heat regulation of the infant is eliminated as far as possible." Results even lower than this, however, may be seen in several instances amongst the data reported in the more recent publications, notwithstand-

ing the lower temperatures employed. A careful scrutiny of the several tables has failed to reveal any relationship between external temperature and the metabolism recorded. Doubtless the infants in the several series of observations were wrapped in different quantities of clothing and bedding necessary to maintain an environmental temperature high enough to induce quiet sleep which was always the aim. Since the notes with reference to this precaution are not very complete, it will be necessary to give special attention to clothing before any final judgment as to the influence of external temperature can be rendered.

In conclusion of this discussion of the factors which may influence heat production in the newborn, emphasis should be placed once more upon the fact attested by several observers that crying is the only normal form of activity which can materially raise the metabolism above the basal level. In the words of our Danish colleague, "as regards the amount of the metabolism, . . . it seems impossible for me to conclude anything else from the tables than that the activity of the infant is the chief determining factor." Hasselbalch goes on to say that even the influence of age has not been demonstrated (in the newborn). While sanction cannot be given to this statement since the publication of Benedict and Talbot's results (see page 635), emphatic assent can be given to his estimate of the muscular factor. The newborn does not shiver. He responds, however, to a drop in external temperature, as he does to hunger, very promptly, by crying, and since this form of exercise is almost his only resort, it serves at once the double purpose of restoring the heat production to an equality with heat loss and of calling the attention of his nurse to his unhappy plight. The importance of conserving the energy resources of the newborn infant by keeping him warm, especially before his natural food is forthcoming, is obvious.

7. Total Energy Requirement of the Newborn.—Thus far we have considered the basal metabolism—i. e., the metabolism of the sleeping infant—and have learned that body weight is nearly, if not quite, as good a measure as body surface, and that length of body (stature) combined with surface (or weight) gives possibly the best measure now available. The newborn up to one week of age requires for maintenance while asleep 1.87 calories per kilogram and hour or about 25 calories per square meter of body surface (Mech). On the 24 hour basis this becomes 45 calories per kilogram or 600 calories per square meter of body surface. The formula of Benedict and Talbot (*b*) $(L \times 12.65 \times 10.3 \sqrt[3]{(w)^2})$, i. e., length in centimeters times a constant times the body surface, as given by Lissauer's formula, is a slightly closer approximation to the average needs. There is a normal variation from this standard of 6 per cent, due to factors (possibly endocrine index) not yet understood.

For the time during which the infant is awake and crying, the requirement, as nearly as it can be estimated to-day, is from 30 to 40 per cent

higher. Since, however, the period of crying continues for the normal newborn rarely more than a few hours at most, the additional allowance of food energy should not be computed on a 24-hour basis, but an attempt should be made to estimate the total period of crying.

The energy allowance for growth cannot yet be estimated with any accuracy. In general it may be stated only that any energy left over after the basal and activity metabolism are provided for will be available for growth, since, so far as we can see at present, no allowance is necessary for dynamic action or for fluctuations of external temperature.

It would appear from the foregoing that an energy supply of 2.5 calories per kilogram per hour or 60 calories per kilogram and 24 hours, will amply cover the maintenance requirement of newborn infants who are not more than normally active. Any intake beyond this amount may, it is presumed, be counted upon to furnish materials for growth. Further study of the "growth quota" in infants of this age, however, is very much needed.

G. Energy Metabolism from Two Weeks to One Year of Age

The energy metabolism of infants over two weeks of age has been much more extensively studied. Beginning with the fragmentary observations of Forster in 1877 down to and including 1920, not less than a score of important researches have been published on the normal child. (Birk and Edelstein, Howland(*b*), Rubner and Heubner(*a, b, c,*), Schlossmann and Murschauser (*a, b, c, d*), Bahrdt and Edelstein, Frank and Wolff, Murlin and Hoobler, Niemann(*a, c*), Bonniot, Saint-Albin, Variot and Lavialle, Hoobler(*b*)). These fall into two groups according to the method of observation adopted. The earlier researches by the indirect method were made for the most part upon a few individuals, but these were studied very exhaustively with a view to account for all of the food ingested. The later researches by the indirect method and all the observations upon normal infants by the direct method have sought rather to establish standards of metabolism with which abnormal or pathological cases could be compared. Consequently a considerable number of subjects have usually been employed. Several of the investigators have selected from their own cases those whom they consider normal. In the case of some others it has been necessary to select from the published tables whom the authors describe as of normal weight for age.

1. Respiratory Quotient.—Very little need be added to what was said under this heading for the newborn. Carbohydrate is the food which influences the quotient most. Soon after a feeding of milk, whether breast or cow's milk, the quotient will be found higher than just before, provided

the feedings are two hours or more apart, and if easily assimilable sugars are added to the milk the quotient will be even higher. For example, an infant four months of age was given a dextri maltose formula and the respiration experiments were begun on different days at successively longer intervals from feeding with the following results:

Time After Feeding	R. Q.
18 minutes	0.79
33 "	0.82
1 hour 30 minutes	1.00

From this point the quotient usually falls progressively (see page 631). Benedict and Talbot's (*a*, *b*) data show many cases like the following:

Case	Time After Feeding	R. Q.
F. B.	6 to 7½ hours	0.80
	20 to 22 "	0.78
	25 to 27 "	0.73
R. E.	6 to 8 "	0.82
	18½ to 21 "	0.74
	24 to 26½ "	0.72

Schlossmann and Murschauser(*d*), however, often found quotients as high as 0.84 and 0.85 as much as 18 to 20 hours after last food. No details regarding the composition of the food taken at the last feeding are given.

The fact that the respiratory quotient is higher soon after a meal (and progressively falls from a point which may be placed at 1 to 2½ hours thereafter depending on the formula) does not denote accelerated heat production, for it will be remembered that carbon dioxid has a lower heat value when the quotient is high than when it is low (see page 567).

Another reason why an ordinary feeding of milk does not raise the heat production in an infant is the interesting fact first recognized by Rubner that protein retained for growth does not raise the heat production. In truth one can say that any foodstuff retained for growth does not raise the heat production. It is only when a surplussage of digestive products enter the circulation that oxidation of them is accelerated by adding more fuel to the fire or by stimulating the intracellular processes. In the infant or any other stage of active growth (pregnancy or convalescence) the materials entering the circulation are retained with greater avidity by the cells and therefore are not exposed to the destructive oxidations to the same extent as in the normal adult. Hoobler(*b*) has made this point as regards protein an object of special study in an infant, with the following results:

	Protein Ingested, Gms.	Protein Destroyed, Gms.	Protein Added to Body, Gms.	Calories of Metabolism
Period I	33.1	18.0	15.1	363
Period II	43.3	18.9	24.4	363

2. Basal Metabolism.—Three different observers have attempted to secure the metabolism of the infant while fasting. Rubner and Heubner compared the metabolism of a breast-fed infant 5½ months old and weighing nearly ten kilos while on a full diet four days with his metabolism on the fifth day when he received only tea instead of the breast milk. The metabolism on the day of starvation was even higher than the average of four days on food.

Two objections may be urged against this experiment: First, that no graphic record was obtained to prove that the infant was just as quiet on the starvation as on the food days. It is almost unbelievable that such should be the case. The second objection is that caffein is known to increase metabolism and there is every reason to believe that the closely related *thein* might have a similar effect especially upon an unhabituated infant. Howland(*b*) tried an experiment in fasting in much the same way with an infant three months of age, and weighing 4.65 kgm., giving tea and saccharin instead of ½ milk and 5 per cent milk sugar which had been the regular food. The result was the same: namely, that the metabolism was not quite as low even when the child was known to be asleep as while sleeping after a feeding. The first objection urged against Rubner and Huebner's experiment would not, therefore, seem to apply, although a graphic record giving proof that sleep while fasting was just as peaceful as after feeding would be required to make the matter wholly convincing. The second objection has not been removed. Schlossmann and Murschauser(*a*) kept careful and continuous notation of the repose of their infants, and determined the metabolism repeatedly on the three different female infants from 87 to 180 days of age 18 hours after last food. All received tea and saccharin which the authors used habitually to soothe their subjects to sleep. The average minimal metabolism of the three was 12.22 gm. CO_2 and 11.02 gm. O_2 per square meter (Meeh) of body surface per hour, or 859 calories per square meter and 24 hours.

It will be apparent from this recital that the whole question of basal metabolism is complicated on the one hand by the difficulty of securing perfect repose without any immediately preceding meal and on the other hand by the question of age. None of the researches yet reported have fulfilled in a wholly convincing manner the conditions now recognized as necessary to secure the absolute basal metabolism of infants. We must be content for the present, therefore, to speak of the lowest metabolism obtainable under the various circumstances as the "minimal metabolism." As landmarks of progress in this direction, the brief table on page 643 may be borne in mind.

It is somewhat hazardous to compare the results of different authors obtained on different subjects by methods which are not strictly alike; but the results suggest, if they do not prove, that the stage of digestion as well as the age of the infant is a factor which must be reckoned with in at-

tempting to arrive at truly basal conditions. The environing temperature was different in the groups of cases cited, but the fact that quiet sleep was

TABLE 30

AVERAGE MINIMAL METABOLISM OF NORMAL INFANTS

(All sleeping or nearly quiet)

Authors	Condition	Cases Averaged	Age, Months	Calories per Sq. M. (Meeh) and 24 Hours
Schlossmann and Murschauser (*a*)	Fasting 18 hrs.	3 (S, P, L)	3-6	859
Benedict and Talbot (*a*)	Post-* Absorptive (?)	6 (E.F., E.R., A.S., R.A., N.D., B.F.)	2-3	809
Murlin and Hoobler	½ to 3 hrs. after feeding	4 (A.S., W.I., E.H., E.N.)	2-3	843
Benedict and Talbot (*a*)	Post-Absorptive	2 (E.G., P.S.)	10 and 12	983
Murlin and Hoobler	¼ to 5 hrs. after feeding	2 (C.M., W.S.)	10½ and 12	1104

* No details given by authors for three of these infants.

induced may be accepted as proof that the clothing was properly adapted to the temperature of the chamber.

We pass now to a consideration of the two factors just mentioned: namely, (1) the dynamic action of food, and (2) the influence of age upon the metabolism.

3. Dynamic Action of Foods in Infants.—It will be seen later that the average energy metabolism of the sleeping infant from two months to one year of age is about 2½ times that of the adult on the basis of weight. This means that the alimentary tract of the infant must be at least two and one-half times as active as that of the adult in order to supply to the circulation the materials necessary for combustion. Added to this is the requirement for growth. It might be expected *a priori*, therefore, that the proportionately more rapid streaming of materials into the blood (see page 605) would set up a greater dynamic effect in the infant than in the adult. The evidence to date, however, is that the reverse is true.

Rubner and Heubner (*b*) were of the opinion that they had demonstrated a dynamic effect of cow's milk when they found in their second study a higher heat production in an artificially-fed infant of 7½ months than in their first breast-fed infant of nine weeks. Using the latter as a basal experiment, they calculated that a diet of cow's milk containing 44 per cent more than the maintenance requirement of energy had raised the metabolism in the former 9.7 per cent. The difference in the ages of the two infants together with the absence of certainty that the second

infant was not more active than the first wholly invalidates their conclusions.

The dynamic effect of protein in the metabolism of an infant was first proved by Howland (*b*). Adding 4 grams of nutrose (containing 14.25 per cent nitrogen) to each of three previous feedings increased the metabolism of his first subject, three months of age, 10 per cent. Adding 30 grams to the food of his second child of 7 months raised the metabolism 26 per cent.

TABLE 31

DYNAMIC EFFECT OF PROTEIN (Howland)

Date	Weight	Food	Calories per Hour
1911			
Feb. 23.....	4.32	⅔ Cow's Milk, 5% Milk Sugar	15.35 Sleeping entire time
Feb. 25.....	4.32	Same, + 30 gm. Nutrose	19.31 " " "
		Difference	3.96 Cal. or 26%

Murlin and Hoobler saw a similar effect from changing to a richer protein formula the diet of an atrophic infant three months of age. The nitrogen in the urine rose in response to the greater intake of protein and the heat production was increased more than two calories per hour. The child slept throughout, but made more frequent readjustment movements after the high protein feeding. Hoobler(*b*) followed up this subject independently and demonstrated a much higher metabolism by feeding progressively higher and higher protein formulas. The following comparison of the periods on low and on high protein diets summarizes his results on a single subject.

TABLE 32

DYNAMIC EFFECT OF PROTEIN (Hoobler)

No. of Hrs.	Food	Degree of Repose	Distribution of Calories	Calories Produced		Increase Per Cent
				Per Hr.	Per Sq. M. 24 Hrs.	
5	Low Prot.	Sleeping	P, 12.2%; F, 26.4%; CH, 61.4%	10.78	893	
10	High Prot.	Sleeping	P, 40.2%; F, 18.7%; CH, 41.1%	12.74	1120	25.4

The highest dynamic effect of milk protein ever recorded was obtained on this child on the twelfth day of the special feedings when the amount of protein (in the form of albumin-milk) in the 24 hours food was 43.3 grams compared with 9.9 grams in the basal diet. The dynamic effect in absolute terms was 108 calories for the 24 hours, or 42.4 per cent!

The dynamic action of fat seems to be proved by the following observations made by Niemann(*a*) on a normal, though at the time underweight,

child four weeks of age. In one period of four days when the food contained 127 calories from protein, 105 from fat, and 168 from carbohydrate, or 400 calories in all, the average daily metabolism was 521 calories or 1337 calories per square meter of body surface (Meeh). In the following period of five days the food contained 145 calories from protein, *368 from fat* and 177 from carbohydrate or 629 calories in all. The heat production averaged 569 calories per day or 1443 calories per square meter. An increase of 70 per cent in the energy intake (largely fat) increased the metabolism 10 per cent. Niemann observed a similar effect of increasing the fat in the food of an atrophic infant 22 weeks old. Helleson(*b*) determined the resting metabolism of a normal infant five months old and found that when a part of the carbohydrate of the diet was replaced by an isodynamic amount of fat the heat production was increased 8.3 per cent. Schlossmann made a similar substitution in kind though not in amount and observed an increase in the metabolism of fifteen per cent.

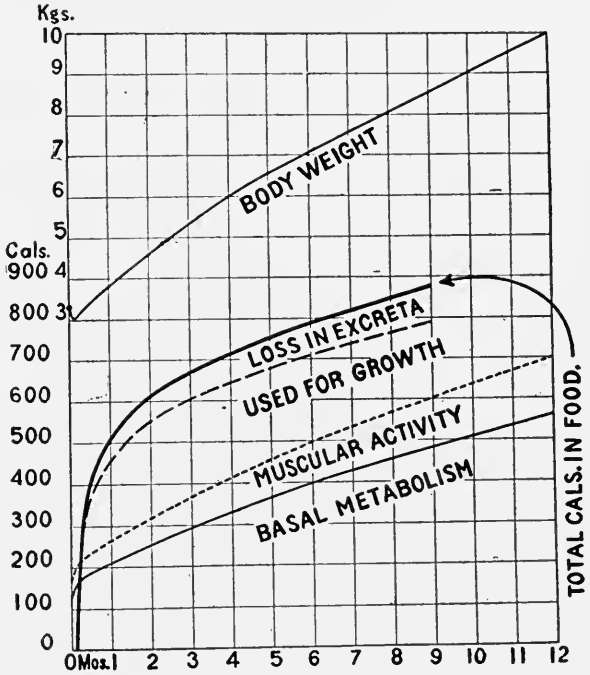


Fig. 37. Metabolism During First Year of Life (Talbot).

The writer is not aware of any experiment establishing the dynamic action of carbohydrate in infants.

The evidence of dynamic action thus far applies only to surplus food. There is no satisfactory evidence that an ordinary feeding given at the time when the infant is naturally ready for it raises the metabolism at all. In the first place the difficulty of securing perfect repose when the infant is hungry has thus far foiled all efforts to get a clean-cut contrast before and after an ordinary feeding. Although Schlossman states in one place that the effect of a meal may be discerned as long as 18 hours afterward, yet as already noted (page 642) neither Schlossman and Murschauser nor Rubner and Heubner nor Howland were able to demonstrate a lower metabolism in fasting. Benedict and Talbot likewise assert that

in some instances the heat production (based on carbon dioxid) in their subjects twenty-one hours after food was slightly "greater even in periods of complete muscular repose" than immediately after food.

4. Influence of Age on Basal Metabolism.—Basal metabolism is the term used to describe the fundamental requirements of the body for energy when it is resting, fasting, and kept comfortably warm. It is the lowest normal metabolism. With the infant this lowest metabolism will always occur during sleep and at that distance from feeding time just preceding the point where hunger becomes so acute as to induce crying or some other form of activity.

In connection with the dynamic action of food we have chosen to speak of the lowest metabolism yet attained as the minimal rather than the basal metabolism; for we have yet to learn of the details of this subject. However, the minimal metabolism ordinarily seen in the infant, i. e., the sleeping metabolism of the recently fed infant, cannot be much greater than the basal metabolism if food really exercises so small an influence on total heat production as it seems to. We shall not go far wrong then in speaking of the minimal metabolism observed in infants of different ages as the true basal.

Benedict and Talbot first demonstrated the influence of age on the basal metabolism per unit of area, although not recognizing the fact, in the following table:

TABLE 33

HEAT-PRODUCTION PER SQUARE METER OF BODY-SURFACE (Meeh Formula) FOR NORMAL INFANTS

Subject	Body-Weight Without Clothing, Kg.	Height, Cm.	Age	Experimental Days	Periods	Heat per Sq. Meter of Body-Surface (Meeh) Cals.
M. D.	3.99	..	17 days	2	4	656
L. L.	5.13	57	2½ mos.	10	13	759
B. D.	4.90	58	2 mos.	2	4	802
M. C.	6.17	63	4 mos.	3	7	837
L. R. B. ...	5.99	64	4 mos.	4	11	844
E. G.	9.37	74	10 mos.	3	5	907
R. L.	7.58	71	8½ mos.	5	8	991
P. W.	7.11	64	7 mos.	2	5	998

The next year Murlin and Hoobler brought together their own data from normal infants and those of Benedict and Talbot published in their second paper and conclusively showed that both on the basis of surface area and weight the metabolism of infants above six months of age is significantly higher than that of infants four months and less. The results are condensed in the following table:

TABLE 34

BASAL HEAT PRODUCTION FROM TWO MONTHS TO ONE YEAR OF AGE

Months	2	3	4	6	7	9	10-11	12
Cal. per Sq. M. and Hr. (Meeh)	34.7	33.2	36.0	40.2	41.6	41.7	41.8	46.4
Cal. per Kgm. per Hr.	2.43	2.29	2.4	2.56	2.57	2.36	2.34	2.61

H. Energy Metabolism of Children up to Puberty

Logically, as we now see very clearly, everything starts from the minimal or mere maintenance requirement, although historically the order has been quite different. The latest and in many respects the most complete researches have been made upon the basal metabolism. It is proper, however, to see how much had been learned regarding the basal needs from earlier investigations.

The Zuntz school headed by the late N. Zuntz of Berlin had long emphasized the necessity of eliminating the influence of muscular activity and of food if results upon subjects of different size or age were to be compared. Magnus-Levy and Falk, followers of Zuntz, employing the well-known method of Zuntz and Geppert with which important results had been obtained on the influence of muscular work in mountain climbing, in marching, and in the treadmill, on the influence of altitude and on the influence of digestion, undertook in 1899 an investigation on the influence of age on the basal metabolism. The subjects ranged from 2½ years to old age, including eleven boys and nine girls under fourteen years of age. At the time of observation the subjects were all in the *nüchtern* condition, which is Zuntz's term for the absence of digestion, i. e., at least twelve hours since taking food, or, what has been called by others, the "post-absorptive state." The subject lay upon a couch and suppressed all muscular contractions. The Zuntz method as described on page 539 permits of the determination of oxygen absorbed as well as of CO_2 eliminated.

The results upon the group of children mentioned above are presented in Table 35. The respiratory quotient characteristic of the *nüchtern* condition in children is well illustrated in this table. The average is 0.82 for boys and 0.84 for girls. With adults the quotient is quite commonly several points higher for the reason that adults do not consume their store of glycogen quite so rapidly. This is in accord with the well known fact that fasting is much more exhausting for children than for adults. The capacity to handle carbohydrates in the diet is the basis of the craving for sweets among children. The arrangement in Table 35, following that of the authors themselves, is according to weight rather than age. It is apparent at once that the metabolism in both sex groups

TABLE 35

THE GASEOUS EXCHANGE OF CHILDREN * (Magnus-Levy and Falk)

Age, Yrs.	Weight, Kgm.	Height, Cm.	O_2 Consumed		R. Q.	Cal. per Sq. M. and Hr.
			Per Kgm. and Hr., c.c.	Per Sq. M. and Hr. (Meeh) liters		
			BOYS			
2½	11.5	?	585	10.74	0.83	51.9
6	14.5	110.0	552	10.92	0.80	52.4
6	18.4	110.0	457	9.78	0.80	46.9
7	19.2	112.0	476	10.32	0.85	50.2
7	20.8	110.0	478	10.68	0.83	51.6
9	21.8	115.0	407	9.24	0.85	44.9
11	26.5	129.0	374	8.22	0.80	39.4
10	30.6	131.0	377	8.52	0.84	41.3
14	36.1	142.0	313	8.40	0.84	40.7
14	36.8	141.5	301	8.10	0.84	39.3
14	43.0	149.0	308	8.76	0.81	42.1
			GIRLS			
7	15.3	107.0	490	9.90	0.81	47.6
6½	18.2	?	445	9.48	0.81	45.6
12	24.0	129.0	338	7.92	0.92	39.2
12	25.2	128.0	322	7.68	0.84	37.2
13	31.0	138.0	332	8.46	0.89	41.6
14	35.5	143.0	317	8.46	0.82	40.8
12	40.2	?	295	8.22	0.78	39.2
11	42.0	149.0	301	8.52	0.81	41.0

* This table is reconstructed in part from a table given by Tigerstedt in Nagel's "Handbuch der Physiologie," 1909, I, p. 475, and in part from a table in Magnus-Levy's "Physiology of Metabolism," Van Noorden's Handbuch, English ed., Vol. I, p. 268.

decreases as age and weight increase, whether it is estimated on the basis of a unit of weight or a unit of surface. Comparing the basal metabolism of a boy and a girl, on the basis of the oxygen absorption, with adults of middle age, and of old age having approximately the same body weight the following result was obtained.

TABLE 36

GASEOUS EXCHANGE AT DIFFERENT AGES (Magnus-Levy and Falk)

	Age	Weight, Kg.	Height, Cm.	Absolute Amount of O_2	Per Kg.	Relative Amount of	
						O_2 per Kilo.	O_2 per Sq. M. Surface
Girl	13	31.0	138	171.7	5.54	112	111
Woman	49	31.6	134	156.6	4.96	100	100
Old Woman ..	75	30.3	circa 140 (?)	128.6	4.25	86	84
Boy	15	43.7	152	216.6	4.97	110	100
Man	24	43.2	148	195.8	4.53	100	100
Old Man	71	47.8	164	163.2	3.42	75	78

They conclude that children produce more heat not merely for the reason that their superficial area is greater in relation to their weight but more also on account of the increased vital energy characteristic of youth.

Sondén and Tigerstedt in the course of an extensive investigation on the metabolism of children sitting quietly as in school, which will be presented later, obtained results on two boys 11.2 and 12 years of age respectively while sleeping. They found the CO_2 elimination on the basis of surface area (Meeh) 52 per cent higher than that of adults in sleep. While the conditions of these experiments did not exclude the influence of food altogether, they approached the true basal conditions very closely and furnished early evidence of a variation directly caused by a difference in age. The conclusion of these authors agrees with that of Magnus-Levy and Falk that the youthful body in and of itself independently of its smaller size possesses a more active metabolism.

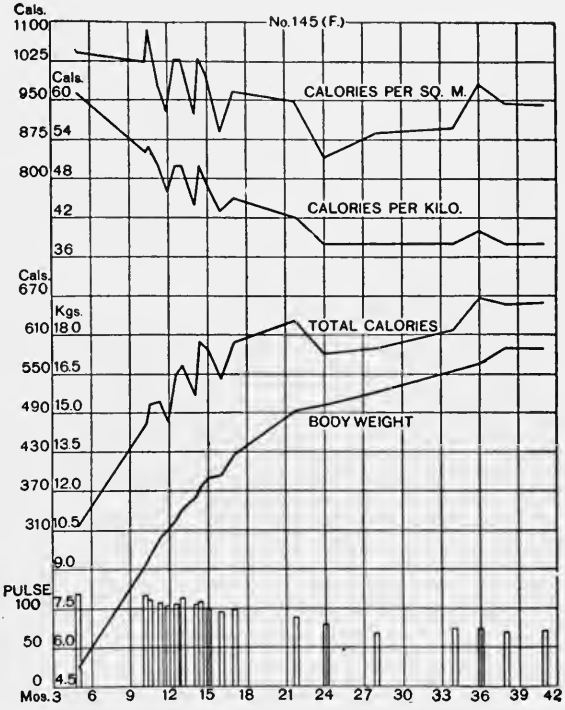


Fig. 38. Body-weight, pulse-rate and basal metabolism per 24 hours of a girl from 5 months to 41 months of age (Benedict and Talbot).

1. Basal Metabolism of Children up to Puberty.—Among the subjects studied at intervals over a long period of time by Benedict and Talbot(*c*) was a girl, designated in their series as No. 145, whose record extends from the age of five months to the age of three years and five months. In all she was placed in the respiration chamber on thirty-one different days and the observational periods of approximately 30 minutes each numbered 4 to 5 daily. The minimal metabolism is given for 25 different days and the accompanying chart represents 19 distinct points in the course of the three years (Fig. 38).

The most rapid growth (as would be expected) is seen in the first half of the time, namely from the 5th to the 21st month. During this time the basal metabolism, calculated to 24 hours (called "total calories" in the

chart) rises nearly parallel with the growth in weight, after which the metabolism rises less rapidly than the weight. It is evident from the curve representing metabolism per unit of weight, however, that the parallelism is only apparent and arises from the fact that metabolism and weight are plotted to ordinates which are not strictly proportional; for the metabolism per kilogram falls from the beginning instead of running horizontally. The level at five months is 60 calories per kilogram and at 24 months it has dropped to 38 calories. From this point onward the curve is horizontal indicating that the progress in growth is equal to the progress in basal heat production. Charted on the basis of a unit of body

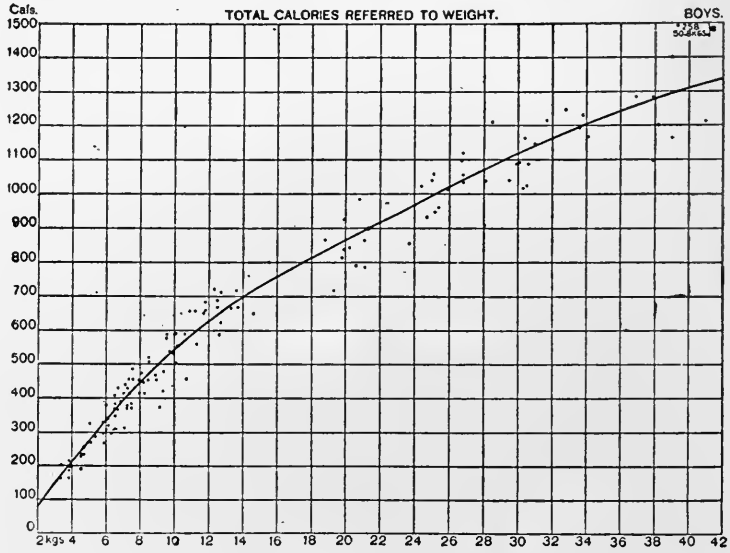


Fig. 39. Basal heat production of boys from birth to puberty. Total calories per 24 hours referred to weight (Benedict and Talbot).

surface (DuBois' linear formula) the general trend again is downward—from 1086 calories at 5 months to 841 at 24 months from which time it rises to nearly 900 calories per square meter at 41 months. Figure 39 gives the progress of the basal metabolism in relation to weight for boys and Fig. 40 the same for girls for the entire series of children studied. The continuous line represents the average; dots individual cases. In the first of these charts it may be seen that the basal metabolism in boys as determined by the most recent observations runs from a little less than 100 calories daily at 2 kilos body weight to 1325 calories at 42 kilos or from about 45 to about 31 calories per kilogram. With girls the curve starts at a slightly lower level at 2 kilos and rises to 1100 calories daily at 32 kilos, or from about 40 to about 34 calories per kilogram. The values obtained by Benedict and Talbot are lower than those obtained by any previous observers except Olin. Curves of the same

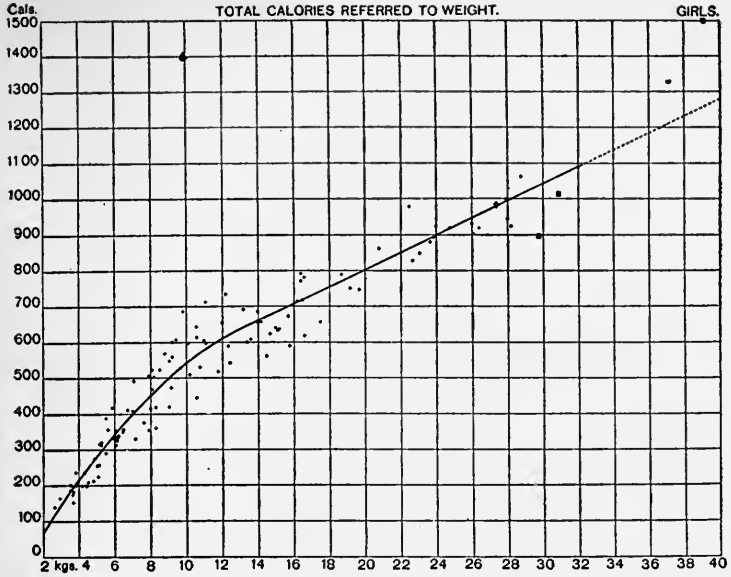


Fig. 40. Basal heat production of girls from birth to puberty. Total calories per 24 hours referred to body weight (Benedict and Talbot).

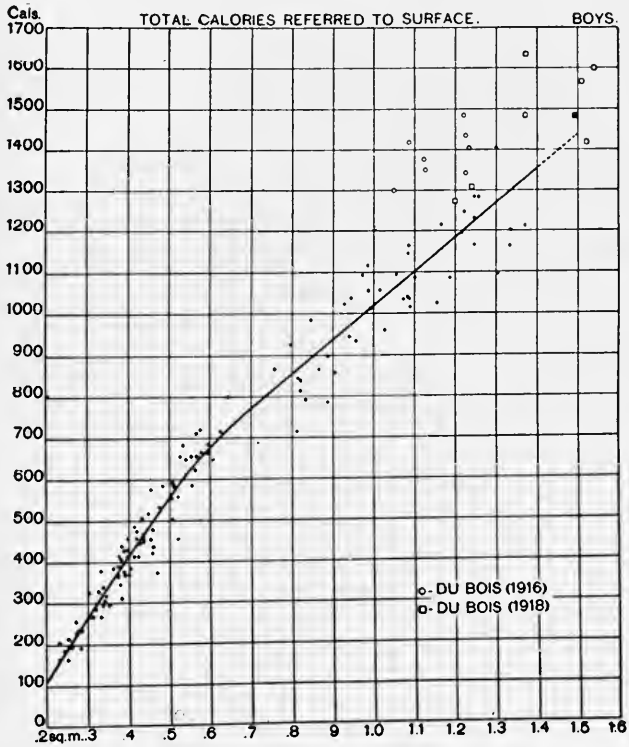


Fig. 41. Basal heat production of boys from birth to puberty. Total calories per 24 hours referred to surface area (Benedict and Talbot).

general character are obtained when the total basal heat production calculated to 24 hours is referred to the body surface (Figs. 41 and 42). The surface area in these observations was calculated from numerous actual measurements according to DuBois linear formula, and a revision of the formula of Lissauer is proposed by derivation of the constant, with which the two-thirds power of the weight should be affected, from the surface as measured. The authors find a slightly closer agreement upon this basis than upon the basis of weight, but persist in their belief that there is no causal relationship between body surface and heat production. This topic has been sufficiently discussed at p. 598 and it may only be reiterated here, that the vastly better agreement between basal heat production and body surface than between this physiological character and body weight, as between individuals of the same species but of widely different size, remains as a challenge to disbelievers. The factor of age must be taken into account as now is definitely established by the work of the several authors described above.

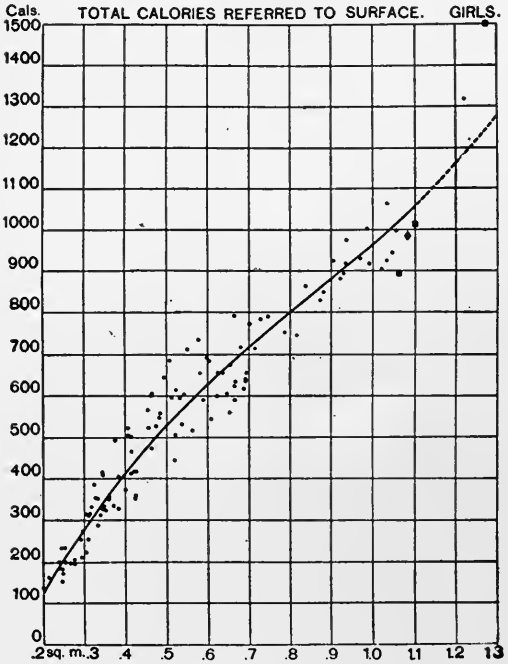


Fig. 42. Basal heat production of girls from birth to puberty, total calories per 24 hours referred to surface area (Benedict and Talbot).

Benedict and Talbot(*c*) find wide variations from their mean curves—from 20 to 64 calories per kilogram and 24 hours for boys and an even wider range for girls; from 650 to 1275 calories per square meter (DuBois linear formula and Lissauer formula modified) per 24 hours for boys, and from 600 to 1350 for girls. The widest variation on both bases for any single age falls in the latter half of the first year, being over 60 per cent for boys and over 65 per cent for girls on the basis of weight; and in the neighborhood of 50 per cent for both sexes on the basis of surface. The variability upon the basis of surface is noticeably less than upon the basis of weight for other ages also.

2. Influence of Sex on Basal Metabolism.—Signs of sex difference in metabolism appear in the very early work of Andral and Gavarret and

of Scharling; but it is not until the classic investigation of Sondén and Tigerstedt that definite proof is furnished. While the conditions of experimentation were not those recognized to-day as essential to demonstrate a basal difference, the authors are very positive in their opinion that under like conditions in the young the CO_2 output both per kilogram of weight and per square meter of surface (Meeh) is considerably greater in males than in females (see page 656). The average difference for their age series (see below, Table 38) is as 140 : 100, "This difference appears to vanish gradually with increasing age until in old age it disappears completely."

DuBois(*a*) first drew attention to a probable difference of actual basal

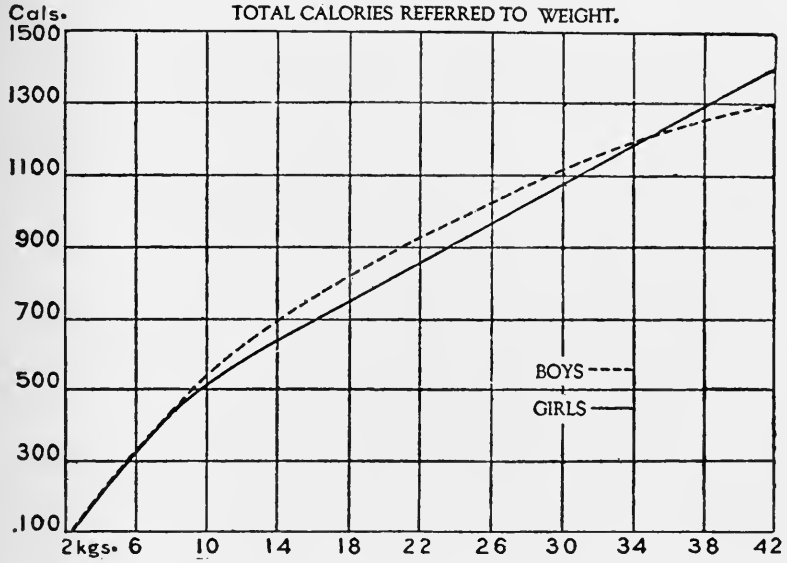


Fig. 43. Comparison of basal heat production of boys and girls per 24 hours referred to body-weight (Benedict and Talbot).

metabolism between the sexes in children (Fig. 35, p. 613) upon the basis of the observations of Magnus-Levy and Falk, who did not themselves recognize such a difference. Its demonstration, however, is due to Benedict and Talbot(*c*). They find that the absence of a sexual difference for the very young infant (p. 635), "persists until about the weight of 11 kgm., but that frequently there is a tendency for the boys to have a somewhat higher metabolism (average) than girls of the same weight" (Fig. 43). On the basis of surface they find that the two sexes remain at essentially the same metabolism (average) until the surface reaches 0.48 sq. M. (DuBois). "From this point the line for the boys rises above that for girls and there is no evidence of a tendency for the two lines to cross later."

a. *Influence of Puberty.*—Andral and Gavarret maintained that with boys the carbon dioxid output suddenly increased at the age of puberty, while with girls it just as suddenly ceased to increase at this critical point. Sondén and Tigerstedt give the following comparison of the total CO_2 output for different age groups using that of a man 57 years of age as 100.

9-12 years	98
13-19 "	126
22-25 "	111
34-44 "	105

The combustion in the body of male individuals from 13 to 19 years of age is therefore greater than that of younger or older individuals of the same sex. This coincides with the period of most rapid growth in length (15th year) and the most rapid growth in weight (16th year).

In a remarkable series of observations on 200 boys ranging from 9 to 19 years of age Olin(*a*) thought she had found, in agreement with Sondén and Tigerstedt, that the CO_2 output whether as total elimination or on the basis of body surface shows a distinct elevation for the age of puberty (14-16) above the general trend of the metabolism for the entire group. Her table given on p. 655, however, does not appear to bear out this conclusion.

The first work carried out on the same youths just before and just after the attainment of sexual maturity was that of Olmstead, Barr and DuBois. Eight normal boys were studied in the respiration calorimeter when they were twelve and thirteen years of age and again two years later when they fourteen and fifteen years of age. On both occasions the boys were placed in the respiration chamber four or five hours after a very light breakfast, which has been shown with adults to leave the basal metabolism unaffected, and were observed for two or three consecutive hourly periods while lying quietly, but for the most part awake. In the first series of observations the basal metabolism was found to be 25 per cent higher than the adult level per unit of surface (linear formula), while in the second after puberty had been definitely established in four of the eight subjects the metabolism was on the average only 11 per cent higher than the adult level. Benedict and Talbot very properly criticise these observations as failing to establish definitely by a sufficient number of observations the true basal, and point out that if the quieter periods of the first series be selected the metabolism is very close to that found in the second series. It might be urged further that there were at the time of DuBois' observations scarcely a sufficient number of basal experiments in the literature at ages preceding and following the ages of his subjects to warrant the inference of a distinct rise in metabolism of the prepubescent age above that of adjacent ages. Benedict and Talbot in a few scattered observations on boys and girls of prepubescent age find no such increase but they admit that their experiments are not yet sufficient in number to warrant a definite conclusion.

3. The Influence of Muscular Activity in Children.—The extensive

observations of Sondén and Tigerstedt at Stockholm, of Rubner(*g*) at Berlin and of v. Willebrand at Helsingfors in contrast with the very low if not actually minimal values obtained by Magnus-Levy and Falk at Berlin, by Olin at Helsingfors and by the Boston workers, furnish some very interesting, though as yet very incomplete, data on the effects of moderate muscular activity.

The resting and post-absorptive rate established by Magnus-Levy and Falk have been discussed above and while the average line established by them lies considerably above that of Benedict and Talbot, their results lie within the range of variability given by the latter authors. So also do those of Olin, notwithstanding that her subjects were studied in the sitting position. They were placed in the apparatus individually, usually in the morning after a light breakfast. The results are summarized in the following table.

TABLE 37

METABOLISM OF BOYS SITTING VERY STILL (Olin)

No. of Subjects	Average Age	Average Height	Body Surface (Meeh) sq. M.	CO_2 per Kgm. and Hr.	Heat Production per Sq. M. and Hr.*
4	9	35.9	1.299	0.425	34.1 Cal.
15	10	31.4	1.217	0.505	37.9 "
14	11	36.1	1.327	0.492	39.3 "
27	12	38.1	1.396	0.372	37.5 "
26	13	43.1	1.573	0.452	35.7 "
22	14	49.6	1.726	0.425	35.3 "
19	15	52.9	1.805	0.412	35.3 "
18	16	59.2	1.948	0.399	35.0 "
9	17	55.4	1.864	0.385	33.5 "
7	18	65.6	2.086	0.359	32.8 "

* Assuming a R. Q. of 0.85 i. e., Heat-value of CO_2 of 5.721 Cal. per liter.

In calculating the surface area by Meeh's formula the constant 12.205 was used by Olin for boys under 13 and 12.847 for boys over that age. The heat production in relation to surface area calculated by the writer upon the assumption of a R. Q. of 0.85 are very close to those ordinarily obtained upon adult subjects under the conditions usually accepted as basal (see page 610). It has recently been shown that a person propped up in a semi-reclining position may have a metabolism even lower than when lying flat in bed. These results by Olin seem to signify that young persons may be induced to sit quietly enough to exhibit a metabolism even lower (?) than when lying down. It would seem that Olin's subjects must have been supported in such a position as to require no muscular tension and that, as in the semi-reclining position in a steamer chair, the diminished pressure of the abdominal organs upon the diaphragm may have lessened the muscular effort of breathing. The results should probably be regarded as representing truly basal conditions.

In strong contrast with these are the figures obtained by Sondén and Tigerstedt upon groups of 6 boys and girls of approximately the same age. The authors state that their purpose was to obtain data which would be of value in determining the ventilation requirements of public assembly halls and especially school rooms. Their subjects were required to sit as still as they would in school, but were permitted to handle and read books and at times to nibble candies and fruits. Their results follow:

TABLE 38

METABOLISM OF CHILDREN SITTING AS IN SCHOOL (Sondén and Tigerstedt)

Average Age		Average Weight	CO_2 per Kgm. and Hour	Calories per Sq. M. (Meeh) and Hr.*
Years	Months			
		BOYS		
7	10	20.1	1.149	73.1
9	7	27.5	1.207	83.1
10	6	30.2	1.106	78.6
11	5	31.6	1.063	76.7
12	6	34.1	0.997	72.1
13	10	44.5	1.000	75.0
14	6	45.3	0.960	74.2
		GIRLS		
7	10	21.8	1.133	74.1
9	11	26.6	0.850	57.8
11	2	31.0	0.845	60.6
12	2	36.2	0.743	56.1
13	4	39.5	0.696	51.4
14	0	44.3	0.661	50.7
15	2	48.6	0.562	44.5

* In view of the fact that the children of this series were permitted to eat candy and fruit at times while in the respiration chamber a R. Q. of 0.90 is assumed, i.e., the CO_2 is given a heat value of 5.471 Cals. per liter.

The heat production here is calculated upon the assumption of a R. Q. of 0.90 employing the values for CO_2 given by the authors upon the basis of a square meter of surface. The results are nearly double those obtained by Olin. Benedict and Talbot have calculated the heat production per kilo and 24 hours of these subjects on the assumption of a R. Q. of 0.90 and these values are shown for comparison upon a chart (Fig. 44) prepared by them to exhibit the basal metabolism according to several authors. The average distance of the individual points designated as the "active subjects of Sondén and Tigerstedt" above the continuous line representing the average basal may be taken as approximating the activity metabolism occasioned by sitting at a desk reading a book and making such minor movements as a well-behaved child in school would make during study periods. This amounts to fully 30 calories per kilogram and 24 hours. Table 38 shows a very marked difference between boys and girls which is

even greater than the difference in basal metabolism between boys and girls (Fig. 43) of the same age. This is due to the greater degree of composure readily induced in girls of the adolescent age.

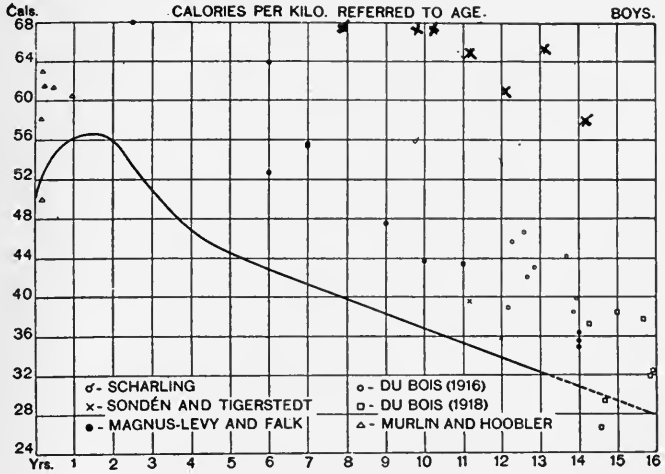


Fig. 44. Basal heat production of boys from birth to puberty (continuous line according to Benedict and Talbot). x x x x Active cases of Sondén and Tigerstedt. Total Calories per kilogram referred to age.

Sondén and Tigerstedt give the following values for two of their boys during sleep:

Boy of 11 yrs. 3 mos.—14.09 gm. CO_2 per sq. M (Meeh) and hour.
" " 12 " —13.78 " " " " " " " "

From which we may derive the following heat production on the assumption of a R. Q. of 0.88:

Boy of 11 yrs. 3 mos.—35.1 cal. per Sq. M. and Hr.
" " 12 " —34.3 " " " " " " "

Boys of the same age in school showed a heat production of fully twice as much (Table 38).

Von Willebrand's observations were made upon boys from 9 to 14 years of age in the apparatus used by Olin. They were confined for the entire 24 hours, taking all three meals in the apparatus. They went to bed at 8 to 9 P. M. and rose in the morning about 6 o'clock. In some instances the subjects slept for a short time during the day. The difference between waking and sleeping metabolism for four individuals is shown in the following table somewhat modified from one given by Benedict and Talbot(*c*).

TABLE 39

METABOLISM OF BOYS AWAKE AND SLEEPING (Von Willebrand)

Name	Age, Years	Body Weight, Kgm.	Cal. per Sq. M.[7] and hour	
			Awake	Asleep
Veikko	9	25.9	57.8	27.3
Viktor	10	30.8	49.0	22.0
Julius	13	34.1	47.6	24.9
Silo	14	36.5	38.9	20.4

[7] Meeh's formula using 12.205 for the first two boys and 12.847 for the second two. Heat is calculated from the CO_2 assuming an R. Q. of 0.83.

Rubner's experiments were made upon two brothers, one fat and one thin, the sons of parents of slender means and therefore not likely to be overfed. They were confined for about 22 out of the 24 hours in the respiration chamber, ate and slept there and during waking hours were permitted to move about, even walking some. The following summary of the results are given by Lusk(*h*).

TABLE 40

METABOLISM OF A FAT AND THIN BOY (Rubner, after Lusk)

	Age, Years	Weight, Kgm.	Heat Production		Per Sq. M. 24 Hrs.	(Meeh) per Hr.
			Total for 24 Hrs.	Total Kgm. and 24 Hrs.		
Fat boy	10	41	1786.1	43.6	1321	55.0
Thin boy	11	26	1352.1	52.0	1290	53.7

The last column may be compared with the results of v. Willebrand (Table 39) and those of Sondén and Tigerstedt (Table 38).

A most interesting phase of the activity metabolism in children, namely, the muscular efficiency as compared with adults, has never been studied. Nor has any attempt been made to estimate the actual energy expenditure of an active child for the entire 24 hours. How much the values just given for boys who were permitted to move about to a limited extent in the respiration chamber falls short of the actual daily requirements with its large quota for growth may be gained from the following chart taken from Lusk(*j*) (Fig. 45).

I. Energy Metabolism of Old Age

In modern times the energy metabolism of old age has been studied by three sets of observers. Magnus-Levy and Falk studied by means of the Zuntz-Geppert method five old men and seven old women. One of their tables has been reproduced on page 648 where comparison is made between the metabolism of a boy and a girl and of middle aged subjects

of approximately the same body weight with two of their aged subjects. Aub and DuBois determined the basal metabolism of six old men between the ages of 75 and 85 years. The authors describe their subjects as "in good condition and fairly well nourished, though on plain and somewhat scanty diets. Considering their ages, they were in good health, though most of them suffered from arteriosclerosis, chronic interstitial nephritis and emphysema, which 'normally' accompany advanced years."

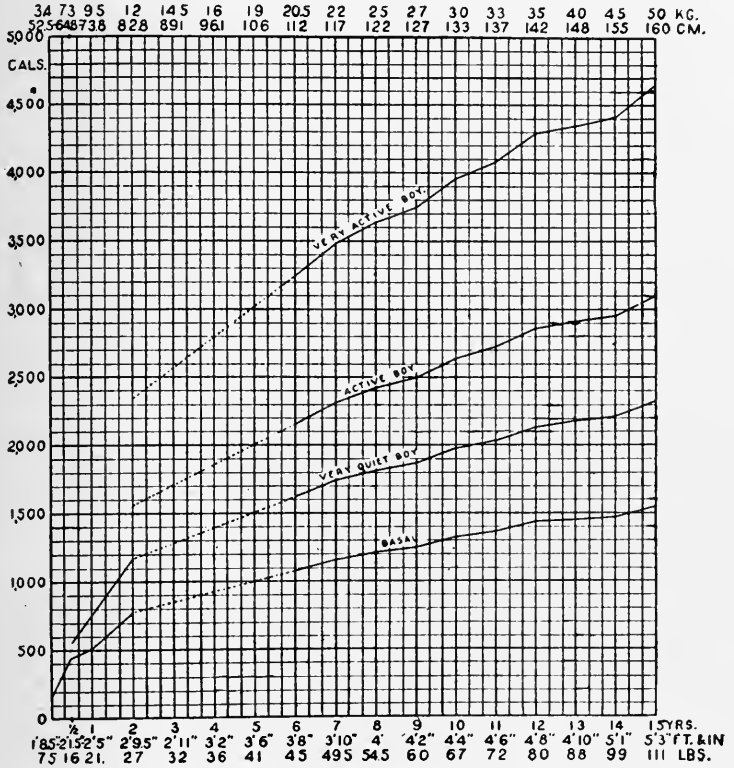


Fig. 45. Metabolism in calories per day of boys from birth to 15 years of age. (After Lusk.)

The average basal heat production was 35.1 calories per square meter (linear formula) per hour, which is 12 per cent below the average for men between the ages of 20 and 50. The respiratory quotients lay between 77 and 86, the average being 81. Since these subjects had been on rather meager fare and were kept in the metabolism ward of Bellevue Hospital for several days before the tests were made, the low metabolism and rather low quotients are in part accounted for by these factors. However, since these conditions accord with the usual routine of life for subjects of very advanced age the metabolism findings are such as would ordinarily obtain.

From the Nutrition Laboratory at Boston are available a few scattered data on the basal metabolism of old people. For example, Benedict (*f*) in a discussion of the factors affecting basal metabolism includes in one of his tables one man 63 and one woman 74 years of age and notes that a person "of advanced years has a still lower metabolism than the person in middle life."

Magnus-Levy observes in explanation of the low metabolism of old age that "the cells of the body lose their thermodynamic powers with old age" and cites the older observations of Andral and Gavarret, Sondén and Tigerstedt and his own work with Falk in support of the view that an old man utilizes less food, not only because his output of work is less, but also because his cells generate less heat during rest. Whatever special causes may underlie the onset of senility physiological old age can only be said to exist when the involution of the various organs takes place gradually and at a proportional rate. In such changes is found sufficient cause for the decreasing metabolism. How low the hour-glass must run before the processes of oxidation must cease or what level of heat production marks the ultra-minimum for the support of respiration and circulation has not yet been disclosed. "And his days were ended and he died, for he was old and weary of life."

SECTION VI

Bacterial Metabolism, Normal and Abnormal, Within the Body . . . . . *Arthur Isaac Kendall*

Introduction—The Significance of Bacterial Metabolism—Bacterial Metabolism—General Relations Between Surface and Volume of Bacteria and the General Energy Requirements of Bacteria—The Influence of Saprophytism, and Pathogenism upon Bacterial Metabolism—Chemical Requirements for Bacterial Development—The General Nature of the Products of Bacterial Growth, Arising from the Utilization of Proteins and of Carbohydrates for Energy—Toxin, Indol and Enzyme Formation—The Specificity of Action of Pathogenic Bacteria and Its Relation to Proteins and Carbohydrates—Quantitative Measures of Bacterial Metabolism, the Effects of Utilizable Carbohydrates upon General Metabolism, and the Elementary Composition of the Bacterial Cell—The Chemistry of Bacterial Metabolism—General Reactions: The Formation of Phenols, Indol and Indican, Amins—Reactions Illustrative of the Decomposition of Proteins by Bacteria—The Effects of Utilizable Carbohydrate upon the Formation of Phenols, Indol and Amins—The Physiological Action of the Aromatic Amins—Summary—Intestinal Bacteriology—General History and Development—The Intestinal Bacteria of Normal Nurslings—Adolescent and Adult Intestinal Bacteriology—Sour Milk Therapy and Bacterial Metabolism—Exogenous Intestinal Infections—Summary and Conclusions.

Bacterial Metabolism, Normal and Abnormal, Within the Body

ARTHUR ISAAC KENDALL

CHICAGO

A. Introduction: The Significance of Bacterial Metabolism

That remarkable chapter in the history of the development of the Science of Medicine which treats of the relations of microörganisms to the causation of specific disease in man has exposed an entirely new and extraordinarily fertile field for study and for speculation.

The first two decades of this era were greatly enriched by the isolation and identification of microbes which were shown to be etiological agents in some of the most formidable infections of mankind. The second decade of this period also witnessed the beginnings of specific bacterial therapy. The brilliant investigations of Von Behring, Kitasato, Roux, Yersin, Smith and others, upon the soluble toxins of diphtheria and tetanus bacilli, and the preparation of their specific antitoxins, seemed to prepare the way for a universal antitoxic therapy which should be efficacious in all disorders of microbic causation.[1]

Time has shown, however, that antitoxic therapy is limited to a very few specific diseases. The development of the field of Immunology by Ehrlich, Metchnikoff, Bordet and their followers, and the elucidation of the nature of the complex reciprocal relationships between host and parasite, which comprise the phenomena of infection and of resistance to infection have shown the basis for antitoxic therapy very clearly, and the limitations which surround it. These studies also indicate very definitely that entirely new procedures must be established to combat those microorganisms for whose pernicious activities no antitoxins can be prepared.

The third decade of medical bacteriology has been endowed with greatly improved methods of culture. These have led to the discovery of many incitants of infection that had eluded the earlier attempts at isolation. The rapid development of the Science of Serology, and the defini-

[1] Von Behring: Die Blutserum-therapie, Leipzig, 1892.

tion of the limits surrounding the uses of vaccines for therapeutic purposes, are also significant events of this decade. The preparation of specific serums, begun in this period, represents as yet an immature phase of bacteriotherapy, but it is a most promising field for further study.

Progress up to the present time in medical bacteriology, therefore, has been chiefly along diagnostic lines, both with reference to the isolation and identification of the etiological agents of specific microbic diseases, and with reference to the recognition of serological reactions in infected individuals. Indeed, with the exception of those few bacteria to whose soluble toxins specific antitoxins have been prepared, the advances in the ameliorative and curative aspects of medical bacteriology have been disappointingly limited. Yet this is the most important field of all.

It is quite apparent that a shifting of the point of attack must precede further advances. Diagnostic, or morphologic, bacteriology must give place to dynamic or chemical bacteriology. "It is what bacteria do rather than what bacteria are that commands our attention, since our interest centers in the host rather than the parasite," as Theobald Smith has so aptly said. The application of biochemical methods to the elucidation of conditions which surround the preparation of soluble toxins, and which, therefore, permit of the generation of potent antitoxins is a striking example of the correctness of this dynamic principle: Those same phenomena which influence the formation of toxin in cultures of diphtheria bacilli play a very important part in determining the nature of the significant products formed by other pathogenic bacteria.

It is not without significance that those very procedures which Escherich and the long list of bacteriologists following him have found useful, and even essential for the identification of microbes have their origin and explanation in these bacteriochemical studies of the mode of action of bacteria. In this regard, bacteriology merges imperceptibly into the fields of protein and carbohydrate chemistry.

Also, the explanation for the striking alternations of bacterial types in the alimentary canal in response to dietary stimuli, and for the conditions which surround the production of endogenous, physiologically active bacterial putrefaction products, depends upon the same biochemical principle of bacterial metabolism. The amelioration, or even the rectification, of exogenous and endogenous disturbances or infections of microbic causation in the alimentary canal can be accomplished through the simple and direct application of the same metabolic principle. A new science, that of bacteriochemistry, is gradually forging into prominence. A new field in medical bacteriology is developing. In this new field, certain fundamental principles underlying the metabolism of bacteria, are being exploited in the direct interest of the host. The nature of these principles, their limitations, their relation to bacteria, and to bacterial infections of man, are discussed in the following pages.

B. Bacterial Metabolism

1. General Relations Between Surface and Volume of Bacteria and the General Energy Requirements of Bacteria

Bacteria in common with all living things exhibit two distinct phases in their life history—the anabolic or structural phase, and the katabolic or energy phase. Of these, while no absolutely sharp line of demarcation can always be determined, the manifestations and significance of the latter phase are by far the more conspicuous, inasmuch as the amount of material transformed into energy and heat far exceeds that entering into the body of the organism and the replacements of structural wear and tear, and losses incidental to the formation of enzymes and other essential nitrogenous secretions and excretions.

The bacteria differ quantitatively from the great majority of plants and animals in their disproportionately large ratio between surface and volume. An ordinary typhoid bacillus, for example, has a volume of approximately 0.000000002 cubic millimeter. The surface area of a bacterium of this size is nearly 0.00001 square millimeter. Inasmuch as the energy requirement of organisms in general varies with the surface area rather than with the volume (Du Bois), it is not surprising to find that bacteria bring about transformations of nutritive material for metabolic requirements considerably greater than their minute size would appear to permit of at first sight.[2]

Bacterial cells exhibit no morphologically definable nucleus,[3] and the complex phenomena attending nuclear division, so characteristic of more highly organized cellular structures, is not a feature of bacterial multiplication. Hence, reproduction among bacteria is mechanically an apparently simple process. It takes place by direct transverse fission, the resulting parent and daughter cells being of approximately equal size.

The rate of increase among bacteria is a geometrical progression which in favorable mediums is theoretically maintained until the accumulation of waste products and other environmental factors imposes a restraint upon the process.

Among the more rapidly growing organisms, as for example the cholera vibrio, successive generations may appear at intervals as frequent as

[2] A man of average figure, 200 cm. long and weighing 100 kg., would have a surface area of about 2.36 square meters. It will be seen that the ratio between weight [or volume] and surface in this instance is much more nearly equal than that of the bacteria.

[3] Bacterial cells are, however, rich in nuclear material. The chemical basis for nuclei probably is quite uniformly distributed throughout the entire cell.

every fifteen minutes. The theoretical descendants of a single microbe after four hours of unrestrained growth would number almost thirty-three thousand. Their combined volumes would be approximately 0.000066 cubic millimeter,[4] but their united surface areas would be nearly 0.33 square millimeter. It is obvious that the amount of structural substance essential for the thirty-three thousand cholera vibrios would be little indeed; the quantity of material necessary to provide the requisite energy for these organisms is relatively very large.

The rapidity of reproduction among bacteria, therefore, furnishes an additional explanation of the magnitude of transformation of nutritive material, which is such a conspicuous feature of bacterial growth. From this viewpoint, the activities of bacteria appear to lie within the realm of colloidal chemistry—the chemistry of surface relations.

The relations between surface and volume of bacterial cells as an explanation of the magnitude of bacterial metabolism cannot be emphasized to the exclusion of the specific activities of individual species or types of bacteria, however. Bacillus proteus and Bacillus typhosus, for example, are of nearly equal dimensions and multiply at nearly the same rate. Nevertheless, the former is far more energetic, under apparently parallel conditions, in its chemical transformations to obtain the elements requisite for energy than the latter.[5] The fact remains, however, that in general, bacteria effect changes in their chemical environment, both in time and amount, greatly exceeding that to be expected from such minute organisms, and the significant aspect of this activity is that associated with the energy phase rather than the structural phase of their metabolism.

2. The Influence of Saprophytism, Parasitism, and Pathogenism upon Bacterial Metabolism

From the viewpoint of mankind, bacteria may be classed for convenience as of three principal groups (Smith, Kendall(*a*)): First, saprophytic bacteria, living upon dead organic material, and usually without significance in a pathogenic way. Their function in Nature is to bring about deep-seated changes in dead organic matter, returning the essential elements, as nitrogen, to the vegetable kingdom as fully mineralized compounds ready for resynthesis into proteins and other necessary organic compounds, by chlorophyll-bearing plants. Secondly, parasitic bacteria, which live upon the body of the host or in channels or cavities in free communication with the exterior of the body of the host. Usually such organisms are endowed with the power of multiplying within the tissues,

[4] The cholera vibrio is approximately equal in size to the typhoid bacillus.

[5] In general, it may be stated that non-pathogenic bacteria are more active chemically than pathogenic bacteria.

in the presence of opposition from the various bactericidal forces of the host, but they lack the power of independent invasiveness. They are "opportunists" with respect to pathogenicity and they are usually secondary invaders because they require some break in the continuity of the skin or mucous membranes to permit of their entrance to underlying tissues. Such an organism is the Streptococcus. Parasitic bacteria do not ordinarily incite epidemics, because they have not perfected a mechanism for escape from the tissues, and as a general rule their excursion into the tissues results in relatively non-specific inflammatory processes, rather than well-defined anatomical lesions.[6] Recovery from an invasion of organisms of the opportunist type does not ordinarily appear to result in a well-defined specific immunity, thus again affording a contrast to bacteria of the progressively pathogenic type.

Finally, the members of a small but formidable group of bacteria are progressively pathogenic, that is to say, they appear to possess the power of independent invasiveness of the body, if they reach a suitable portal of entry in sufficient numbers. After invasion they multiply for a period of time within the tissues of the body in the presence of the opposition offered by the various non-specific lines of defense. They have individually perfected, finally, well-defined mechanisms of escape from the tissues to channels in communication with the outside world, thus providing for escape to other, susceptible hosts, and the perpetuation of the species.

The typhoid bacillus may be cited as illustrative: The organism must reach the small intestine of a susceptible individual, penetrate the mucosa, and enter the circulation. It grows in the tissues and, after a period of time, reënters the intestines from the gall bladder from which it escapes to the environment in increased numbers, or it escapes from the urinary bladder to the outside world.

Thus, it is possible to distinguish a "cycle of parasitism" and a "cycle of pathogenism." The essential factors of the former are—first, for the parasitic microbe to reach the surface of a suitable host, or to reach channels or cavities in free communication with the outside world; secondly, for the microbe to multiply there, and, thirdly, to escape to other, suitable hosts, thus insuring the perpetuation of the species. Penetration of the tissues and growth therein is not a part of this cycle—the microbe cannot escape to the outside, as a general rule, and perishes, although it may overwhelm the host in so doing. Parasitic organisms, therefore, are not progressively pathogenic. The pathogenic cycle is somewhat more complex. The organism must reach a suitable portal of entry to the underlying tissues of the host, actually penetrate into the underlying tissues and grow therein in the face of non-specific and specific opposition. Finally,

[6] Thus, the lesions caused by progressively pathogenic bacteria, as the tubercle, typhoid, or syphilis microbes, are fairly distinctive and characteristic in structure and distribution, contrasting sharply in this respect with the non-specific inflammations induced by streptococci or other pyogenic microbes.

the organism must escape from the tissues in significant numbers to channels in communication with the outside world, and eventually reach other, suitable hosts. Such organisms incite specific epidemics. They are progressively pathogenic from host to host.

It is a striking fact that the evolution of bacteria from saprophytic types through parasitic to pathogenic types has been attended by a marked decrease in the chemical activities of the microbes. For example, the contrast in chemical activity between the powerfully proteolytic members of the saprophytic hay bacillus group, which are without virulence, through the ordinary skin Staphylococcus to the exquisitely fastidious Meningococcus is only equaled by the increased pathogenicity of these latter organisms. Generally speaking, intense chemical activity appears to be incompatible with pathogenicity (Kendall).

The facts adduced thus far relate to general properties of bacteria; they furnish little or no information relative to the specificity of bacteria and of bacterial action. Bacteria, in the last analysis, are "living chemical reagents," as Professor Folin once characterized them, and the specificity of bacterial action is largely, if not almost wholly, a problem of the chemistry of their interchange with their environment.

The ultimate chemistry of bacterial action, particularly that relating to the pathogenic organisms, is as yet unsolved. The formulæ for diphtheria and tetanus toxins, the nature of the poisons of the typhoid and dysentery bacilli, are problems for the bacteriological chemists of the future to solve. Nevertheless, all bacteria of interest or of importance to man exhibit certain rather general relationships with respect to their energy requirements, which are of interest and of increasing importance in the solution of certain problems of medicine. A discussion of these relationships will necessitate a survey of the general phenomena of bacterial nutrition.

3. Chemical Requirements for Bacterial Development:

a—For Structure. b—For Energy.

The cytoplasm of bacteria contains nitrogen, carbon, hydrogen and oxygen, together with other elements in lesser amounts, in about the same proportions as those found in other living cells. The phosphoric acid content is higher than that found in the cells of a majority of higher plants or animals, however.[7] It is obvious that the growth of bacteria in the abstract depends upon the availability of these elements, together with those of lesser occurrence, in proper amounts and in proper combinations. For purposes of discussion, attention will be directed specifically toward

[7] Thus, the ash of Bacillus xerosis contains 34 per cent of phosphorus calculated as phosphoric acid, the tubercle bacillus 55 per cent, the cholera vibrio about 45 per cent.

nitrogen, as an element of great structural significance, and carbon, of peculiar importance as the basis of the energy phase of bacterial metabolism.

a. **Structural Chemical Requirements.**—Bacteria can not multiply in non-nitrogenous media, and the organisms of interest and significance to man derive their nitrogen requirements from nitrogen in combination with carbon, hydrogen and oxygen of the amino-acid complexes—polypeptids, peptones, or proteins. The more fastidious organisms, as the Gonococcus and Meningococcus, require, or at least develop best in, media containing protein but little altered from the state in which it exists in the human or animal body. Others grow very well indeed in media containing less highly organized nitrogen, as for example that of peptone. None will grow in the absence of this element; hence, it may be regarded as an essential structural element. Nitrogen has no energy value, however, for parasitic or pathogenic microbes.

b. **Energy Chemical Requirements.**—Bacteria derive their energy from the oxidization of carbon, in the last analysis, and the state of combination of this element with others—particularly oxygen and hydrogen [as well as nitrogen in proteins and protein derivatives]—determines to a very considerable degree the nature of the products of specific bacterial metabolism. The influence of associative elements upon bacterial metabolism and even the specificity of bacterial action, from the viewpoint of energy, is shown in the following well authenticated series of illustrations:

4. The General Nature of the Products of Bacterial Growth, Arising from the Utilization of Proteins and of Carbohydrates for Energy—Toxin, Indol and Enzyme Formation.

Diphtheria Toxin.—It is well known that the soluble or exotoxin of the diphtheria bacillus is the specific product which makes this organism formidable to man. Diphtheria toxin is also excreted incidentally to the growth of the microbe in plain nutritive broth, which consists essentially of a neutral mixture of peptone, meat extractives, salts and water. In such a medium, the diphtheria bacillus develops rapidly and within a week or ten days the filtrate of this culture medium, freed from all bacteria or other particulate matter, is extremely toxic for guinea pigs. Indeed, 0.025 cubic centimeter of such bacteria-free broth frequently kills 250 gram guinea pigs within four days with very definite specific symptoms and lesions.

Contrast this highly toxic broth with that resulting from the growth of the *same* organism under precisely the same conditions in the *same*

medium to which has been added merely a minimum of 0.5 per cent of glucose. Here the broth is acid in reaction in place of slightly alkaline, but otherwise it appears to be the same (Van Turenhout, Smith, Kendall). Injected into guinea pigs, however, the glucose broth is found to be wholly without toxicity. The simple addition of a small amount of glucose has completely changed the character of the products formed as the result of the growth of the diphtheria bacillus. Lactic and other acids are formed under these conditions, but no soluble toxin.

Indol Formation.—The amount of indican excreted in the urine has long been regarded by some observers (Combe, Bahr) as an index of the intensity of that obscure clinical condition spoken of as "auto-intoxication." Irrespective of the clinical significance of urinary indican, however, the parent substance is indol (Kendall), an aromatic residue of the amino acid tryptophan. In man, indol is produced from tryptophan in the intestinal tract by the action of Bacillus coli, Bacillus proteus, and to a lesser extent by other facultative proteolytic organisms, acting in the absence of utilizable carbohydrates. The absorption of indol from the alimentary canal, its oxidization in the liver, and its excretion and significance are discussed later.

The production of indol from tryptophan by cultures of B. coli, Bacillus proteus, the cholera vibrio or other bacteria can be observed readily in the test tube; the conditions favoring or preventing its formation are easily controlled. Indol appears within twenty-four to forty-eight hours in ordinary sugar-free nutrient broth containing tryptophan, such as that in which the diphtheria bacillus produces toxin. Precisely as the addition of glucose to plain nutrient broth prevented the formation of diphtheria toxin by the diphtheria bacillus, so that addition of glucose to such broth prevents the formation of indol by the colon bacillus, Bacillus proteus and the cholera vibrio. In place of indol and other products of putrefaction, which appear in sugar-free media of the kind described, the addition of glucose so changes the products of metabolism of these organisms that only organic acids—as lactic and acetic—are formed, together with carbon dioxid and hydrogen; in other words, the substitution of utilizable carbohydrate for protein as a source of energy alters completely the nature of the products formed.

The Formation of Protein-Liquefying Enzymes.—Bacillus proteus, the cholera vibrio, and several other parasitic and, less commonly, pathogenic bacteria, form soluble enzymes, much like trypsin in their protein-digestive power, in sugar-free media. These enzymes may be obtained in a reactive state, quite free from bacteria, by filtering the latter away (Fuhrmann). The germ-free filtrate is strongly proteolytic for a variety of proteins, including gelatin, breaking the complex molecule into amino acids and polypeptids.

The addition of glucose to cultures of the cholera vibrio or Bacillus

proteus prior to inoculation [to the extent of 0.5 per cent or more] will so alter the products of growth that the soluble proteolytic enzyme and all other evidences of proteolytic and putrefactive activity are no longer detectable in the culture medium (Kendall and Walker). On the contrary, lactic and other acids indicative of the fermentation of carbohydrates are formed. Here again the addition of glucose in a minimal amount of 0.5 per cent has completely altered the products of growth. In other words, from the illustrations cited, small amounts of glucose prevented the formation of toxin in cultures of the diphtheria bacillus, of indol in cultures of Bacillus coli and Bacillus proteus, and of a soluble proteolytic enzyme in cultures of the cholera vibrio and Bacillus proteus. If space permitted, examples of the sparing action of utilizable carbohydrate for protein as sources of energy might be cited from all fields of bacterial activity, but those herewith presented are illustrative. Additional observations of specific interest are discussed in appropriate sections.

It is worthy of note that a minimum of 0.5 per cent of glucose was specified in each instance. Experience has shown that the diphtheria bacillus can utilize from 0.1 to 0.3 per cent of glucose without producing enough fermentation acid and other products of the cleavage of glucose to inhibit its further growth (Theobald Smith). Under these conditions no toxin is demonstrable until the sugar [glucose] has disappeared. Then toxin begins to form.

Bacillus coli and Bacillus proteus do not form indol in culture media until the utilizable sugar is used up. If the amount of sugar is somewhat less than 0.5 per cent, the products of fermentation incidental to the utilization of it for energy do not inhibit the subsequent development of the colon or proteus bacilli, and the formation of indol proceeds after the glucose is fermented.

Similarly, relatively small amounts of glucose or other utilizable carbohydrate, somewhat less than 0.5 per cent—the limit of tolerance varies somewhat with the strain of the organism—prevent the formation of proteolytic enzymes by cholera, proteus and other bacilli. When the carbohydrate is used up, however, provided the conditions due indirectly to the accumulation of products of fermentation are not too unfavorable, the organisms attack the protein constituents of the medium for their energy, and the proteolytic enzyme makes a belated appearance. It should be emphasized that the presence of glucose, or other utilizable carbohydrate in cultures of cholera, proteus, or other bacteria, which form a soluble proteolytic enzyme, prevents the *formation* of the enzyme in the reactive state. Neither glucose nor any other carbohydrate prevents the action of the mature, reactive proteolytic enzyme when it has been elaborated (Kendall and Walker(*b*)). In other words, when the enzyme is formed in an active state, as for example in sugar-free media, this bacteria-free enzyme

will act quite as readily upon protein media containing glucose as upon protein media from which glucose is absent.

The foregoing illustrations typify a very general property of bacteria, and of other living things for that matter, with respect to metabolism. It has long been a physiological dictum that "carbohydrate spares body protein" (Howell(*a*)), meaning by that that an animal requires a definite, if minimal, amount of dietary protein to maintain the nitrogen equilibrium of the adult organism. This minimal amount of nitrogen is indispensable for the repair of structural wear and tear, and for the replacement of nitrogenous losses in secretions, enzymes and other nitrogen-containing substances, which are of necessity constantly lost to the body. The fuel or energy requirement of the organism, on the contrary, amounting to many times the minimal nitrogen requirement, can be met by the feeding of non-nitrogenous food, as carbohydrate and, to a lesser degree, organic acids or fat.

Bacterial nutrition presents the same fundamental phenomena of structural and energy requirements. The former absolutely requires nitrogen as one element in its make-up, whereas the latter may be satisfied by non-nitrogenous organic substances. Of these, the carbohydrates as a class are of paramount importance, although of varying degrees according to specific characteristics of the organisms under investigation. Precisely as saprophytic bacteria were found to be more energetic cleavers of protein than parasitic and pathogenic bacteria, so the saprophytic types are somewhat more energetic cleavers, both in kind and amount, of carbohydrate than the pathogenic types. Hence, a majority of the progressively pathogenic bacteria, as typhoid, dysentery, diphtheria and many others, utilize the hexoses [especially glucose], but fail to utilize the bioses, as lactose and saccharose. The pathogenic bacteria produce less deep seated changes even in the hexoses than do the saprophytic types. In general, the changes induced by the former result in the formation of lactic and acetic acids, whereas the latter frequently oxidize a not inconsiderable portion of the hexose to carbon dioxid and hydrogen.

Returning to the conditions prevailing in cultures of diphtheria, colon and cholera organisms referred to above, it will be found that plain or sugar-free media offer to bacteria protein and protein derivatives [peptone, polypeptids and amino acids], as the sole source of structure and of energy. The glucose media offer precisely the same protein and protein derivatives for structure—non-nitrogenous substances are not suitable for structure, generally speaking—and, in addition, a choice between this protein or protein derivative and carbohydrate for *energy*. To summarize:

The marked difference discernible between the significant products formed by bacteria in non-saccharine media, where both structure and energy requirements are of necessity obtained from the nitrogenous protein

derivatives, and the absence of such significant products [toxin, indol or enxyme] in the glucose-nitrogenous media indicates the importance of the source of energy as a determining factor in directing the type of action of the microbe.

5. The Specificity of Action of Pathogenic Bacteria and Its Relation to Proteins and Carbohydrates

From what has been stated previously, it would appear that pathogenic and parasitic bacteria produce significant or specific nitrogenous waste products incidental to their utilization of protein or protein derivatives for energy. Thus, diphtheria, typhoid, dysentery, cholera, paratyphoid, glanders, colon, proteus, and many other pathogenic microbes produce specific toxins or other characteristic nitrogenous products in protein environments from which utilizable carbohydrates are excluded.

On the contrary, when in addition to protein utilizable carbohydrates are also available as sources of energy, these same organisms act upon the latter instead of the former, and produce therefrom acidic products, chiefly lactic and, to a lesser extent, acetic acid.

In other words, the simple addition of glucose to cultures of pathogenic bacteria, other conditions remaining the same, brings about a striking alteration of the nature of their metabolic products. In place of toxins, phenols, skatol, and other protein derivatives, specific or characteristic of each individual microbe, all produce innocuous lactic and acetic acids. These formidable incitants of disease in man have become potentially lactic acid bacteria. Grown in glucose media, therefore, the diphtheria, typhoid, cholera and other pathogenic bacteria become the qualitative equivalents of the Bulgarian lactic acid bacillus.[8]

Stated differently, it may be said that the *specificity* of *action* of the vast majority of bacteria pathogenic for man is dependent upon their utilization of protein for energy (Kendall).

Fats play a very minor part in the metabolism of pathogenic bacteria, other than those of the acid-fast group, which includes the tubercle and leprosy bacilli. The effects of utilizable fats are comparable to the carbohydrates rather than the proteins, however, so far as their energy relationships are concerned.

The toxicity of the cellular substance of bacteria is not considered in this connection, nor is it relevant. Available evidence indicates that the cytoplasm of non-pathogenic bacteria, as for example Bacillus prodigiosus, may be many fold more deadly to animals than that of such formidable

[8] It is obvious that a continuous supply of utilizable carbohydrate must be available; when the sugar is used up, provided the organisms are not killed by the products resulting from fermentation, they will at once attack the protein again and generate their specific protein decomposition products.

incitants of disease as diphtheria, anthrax, or typhoid bacilli (Vaughan). The effects of carbohydrates and proteins upon the composition of the cytoplasm of bacteria is discussed in the following section.

6. Quantitative Measures of Bacterial Metabolism, the Effects of Utilizable Carbohydrates upon General Metabolism, and the Elementary Composition of the Bacterial Cell.

It is very evident that there are far-reaching theoretical and practical applications of the theory that the "specificity of action of the vast majority of bacteria depends upon their utilization of protein or protein derivatives for energy." The application of the theory to the domain of medicine is closely associated with the corollary thereof, namely, that the "great majority of pathogenic bacteria become potentially lactic acid bacteria when they are growing in an environment containing carbohydrates or other non-nitrogenous compounds from which they can obtain their energy."

So sweeping an assertion would appear to require more than qualitative evidence for its consideration or acceptance. Fortunately, such evidence is available from several sources.

The chemical basis for the proof of the theory of the sparing action of utilizable carbohydrate awaited the development of methods for the study of metabolism which were applicable to bacterial cultures. Qualitative evidence has long been known, even though it was not appreciated for its full significance.

The very exact micro methods of urine analysis, developed and perfected by Folin and his associates (Folin(*d*)), have been found applicable to the study of nitrogenous metabolism in cultures of bacteria (Kendall and Farmer). The analytical data obtained are as precise as any obtainable for corresponding metabolic studies upon man or animals. Indeed, in some respects they are of greater precision, inasmuch as the total nitrogenous changes induced by various bacteria under varying cultural conditions are always reproducible, since there is neither gain nor loss of nitrogen during the experiment.

The quantitative studies of bacterial metabolism were carried out in precisely the same manner as a corresponding metabolic study upon man or upon an experimental animal. Broadly speaking, the significance of the results is the same for bacteria in either case. The results of these quantitative metabolic studies appear to be very clear cut and definite; they bear out exactly what has been indicated by qualitative observations, namely, that utilizable carbohydrate added to protein culture media does

shield the nitrogenous constituents from utilization for energy. These experiments also demonstrate the very considerable amounts of acid—chiefly lactic and acetic—which appear concomitantly with the utilization of the carbohydrate for energy. In this respect, the sugar-protein cultures contrast strikingly with the purely protein cultures, which become more or less alkaline, due to the gradual accumulation of basic, nitrogenous waste products arising from the combustion of the nitrogenous constituents of the non-saccharine media. The nitrogenous waste products arising from the utilization of protein for structural requirements and structural replacements, although relatively small in amount, were also clearly indicated in these quantitative analytical studies.

A word of explanation of the analogy between the metabolic waste products of man and of bacteria will be required to indicate the parallelism between human [multicellular] nitrogenous metabolism and bacterial [unicellular] metabolism.

It will be remembered that the principal end product of the physiological metabolism of the proteins of the food and the tissues in man is excreted through the kidneys into the urine as urea. Urea is derived, in the last analysis, largely or chiefly from the deamination of amino acids: the ammonia liberated is changed, principally in the liver, to urea.

Ammonia has no energy value and whenever amino acids [protein or protein derivatives] are used in the body for energy, for transformation into glucose, or glycerin, or for storage as glycogen or fats, the ammonia is discarded and changed to urea, unless a deficit of alkali leads to its combination with acids that must be excreted through the kidneys. The excretion of urea is markedly increased when a purely protein diet is provided, and it is greatly reduced when the energy requirements of the body are provided for by a carbohydrate regimen, supplying, however, sufficient protein for structural and replacement needs.

This urea may be regarded, therefore, as of exogenous and of endogenous origin (Folin), the former being influenced largely by an excess of protein above the structural requirements, the latter more specifically associated with structural changes in the tissues and organs. The exogenous urea is greatly influenced by the nature of the diet, being increased when the energy requirement of the body is obtained chiefly by the oxidization of proteins and reduced when the energy needs are derived largely from dietary carbohydrate and fat. The endogenous urea is less variable under proper dietary conditions.

Similarly, bacteria deaminize amino acids prior to their utilization of the remainder of the amino acid molecule for energy. Also, a small amount of ammonia is apparently produced from the utilization of some nitrogenous substance for the structural needs of the bacterial cell. Bacteria have no livers; therefore, so far as is known, they do not excrete urea (Kendall and Walker). Ammonia, which has an analogous origin

in man and in bacteria, is "bacterial urea," and as such it is the best available measure of nitrogenous metabolism.

The "endogenous" ammonia is recognizable when bacteria derive their energy solely from carbohydrates, in a protein-carbohydrate medium. It is of course masked in a purely protein medium where deamination of protein occurs prior to the combustion of the protein for energy, as well as from the structural nitrogenous changes.

The following analytical data are illustrative of the nitrogenous metabolism of several saprophytic, parasitic, and pathogenic bacteria, under parallel conditions:

Briefly, the conditions of experiment are as follows: Plain, nutrient, sugar-free broth, and glucose broth respectively, which differ only in that the latter is reënforced with one per cent of glucose, are inoculated with the same organism under exactly similar conditions, incubated side by side, and examined at the same time for changes in titratable acidity and nitrogenous changes, particularly ammonia formation. Ammonia formation is an index of deamination, associated chiefly with the utilization of the non-nitrogenous residue of the amino acid complex for energy. In media containing glucose in addition to the protein derivatives, the energy requirement is obtained largely at the expense of the non-nitrogenous carbohydrate, which of course does not undergo deamination prior to its energy transformation. Under these conditions the sparing action of glucose [carbohydrate] for protein is obviously manifested by a greater or lesser reduction in the amount of ammonia formed [deamination] in contrast to the amount observed in the corresponding glucose-free medium.

The table on following page also shows the relatively lesser nitrogen change in media induced by pathogenic bacteria than that characteristic of the saprophytic types—as, for example, between Bacillus dysenteriæ and Bacillus mesentericus. This is in harmony with the observation cited above that pathogenic organisms, generally speaking, are less active chemically than the ordinary saprophytic types (Kendall, Sears).

Explanation: In general, it will be seen that all the bacteria studied become alkaline in reaction and form considerable amounts of ammonia in sugar-free broth. Among the products formed, but not indicated in the table, are diphtheria toxin by the diphtheria bacillus, indol by Bacillus proteus and Bacillus coli, a soluble proteolytic enzyme by Bacillus mesentericus, Bacillus proteus and Staphylococcus aureus, and a soluble hemolysin by Streptococcus hemolyticus.

In the glucose medium, all the bacteria produce a relatively strong acid reaction [chiefly lactic and acetic acids] and relatively slight amounts of ammonia, indicating that the major reaction is upon the glucose in place of the protein. Neither toxin, enzyme, hemolysin nor indol is to be found among the products produced from glucose by the organisms.

Ten-Day Observations Organism:	*Sugar-Free Broth*		*Glucose Broth*	
	Reaction:	Ammonia:	Reaction:	Ammonia:
B. dysenteriæ Shiga	— 0.30	+ 4.20	+ 2.80	+ 0.70
B. dysenteriæ Flexner	— 0.25	+ 4.50	+ 2.45	+ 0.70
B. diphtheriæ	— 0.50	+ 3.10	+ 2.80	+ 1.05
B. typhosus	— 0.45	+ 5.40	+ 3.10	+ 0.60
B. paratyphosus alpha	— 0.20	+ 6.30	+ 3.40	+ 1.20
B. paratyphosus beta	— 0.60	+ 7.50	+ 3.75	+ 1.40
B. coli	— 1.00	+ 24.40	+ 4.90	+ 1.05
B. proteus	— 2.00	+ 58.40	+ 3.55	+ 1.40
B. mesentericus	— 0.70	+ 38.50	+ 1.50	+ 2.80
Streptococcus hemolyticus	+ 0.70	+ 1.40	+ 3.50	+ 0.70
Staphylococcus aureus	— 0.75	+ 38.70	+ 3.75	+ 0.70

Legend:

Reaction, — indicates the amount of alkalinity developed in terms of normal alkalai per 100 cubic centimeters of culture.
+ indicates the amount of acidity developed, in terms of normal acid per 100 cubic centimeters of culture, compared with suitable controls.

Ammonia, The figures indicate the number of milligrams of nitrogen as ammonia developed in 100 cubic centimeters of media, compared with suitable controls.

These qualitative and quantitative observations, illustrative of the sparing action of utilizable carbohydrate for protein as a source of energy, together with the significance of this sparing action in terms of important products arising from the use of protein, and their replacement by innocuous compounds when carbohydrate is available, leads logically to the generalization that "the significance of the action of pathogenic bacteria, so far as is known, depends upon their utilization of protein for energy." When carbohydrate is used for energy, the organisms are potentially lactic acid bacteria in terms of their reaction products (Kendall).

The endotoxins, so-called, of bacteria are not considered in this discussion, which deals with the products of growth. It appears to be a fact, however, that carbohydrate influences the composition of bacteria in a striking manner. Thus, Cramer has analyzed the dried substance of bacteria grown upon ordinary nutrient agar, and upon glucose agar of otherwise the same composition, with the following results, expressed in percentages:

ORGANISM:	Sugar-Free Agar			Glucose Agar		
	Nitrogen	Alcohol-ether extractives	Ash	Nitrogen	Alcohol ether extractives	Ash
Pfeiffer bacillus	66.6	17.7	12.56	53.7	24.0	9.13
Bacillus H-28	73.1	16.9	11.42	59.0	18.4	9.20
Pneumobacillus	71.7	10.3	13.94	63.3	22.7	7.88
Rhinoscleroma bacillus	68.4	11.1	13.45	62.1	20.0	9.44

It will be seen that bacteria grown on glucose agar contain nearly twenty per cent less nitrogen, and materially more extractives than those grown on media with the same nitrogenous constituents but without the glucose. The significance of this difference is yet to be determined.

Inasmuch as the immunizing processes are apparently inseparable from nitrogenous substances, however, there may be some relationship between a maximum nitrogen content of bacteria and their antigenic potency, which may play a part in the large field of bacterial vaccines. In this connection, the reciprocal variation of nitrogen and lipoids, clearly suggested in the table, may also be of significance inasmuch as solubility and anti-complementary properties of bacteria appear to be related to the lipoidal content of bacterial bodies (Warden). Whatever the significance of the composition of bacteria may be, it may be stated confidently that the entire series of phenomena outlined above—relating to the sparing action of utilizable carbohydrates for protein in the energy manifestations of bacteria and their effects upon the composition of bacteria even—is of material importance in determining the nature and extent of bacterial action.

C. The Chemistry of Bacterial Metabolism

1. General Statements

The chemistry of bacterial metabolism naturally is divided into two rather distinct phases—the anabolic, or structural, phase, which in point of time occurs first, and the katabolic, or energy phase, which follows the maturation of the bacterial cell.[9] The latter exceeds the former, both with respect to the amount of material transformed and in respect to the significance of the products resulting from the utilization of the various substances for energy.

Generally speaking, the structural or anabolic phase consists of a series of hydrogenic condensations whereby simpler nitrogenous substances, as amino acids or polypeptids, are built into specific proteins; where glycerin and fatty acids are synthesized to fats, and, in association with phosphorus, into nucleins; and where glycogen-like bodies are apparently synthesized from glucose.[10] This phase of bacterial development

[9] It is almost certain that a certain amount of interchange referable to the anabolic phase must take place throughout the period of vegetative activity of the cell. The losses associated with the formation of enzymes and other essential excretions belong in this group.

[10] Considerable evidence has accumulated indicating the possibility of a mutual transformation of glycerin, alanin and glucose through pyruvic acid into the three great types of proteins, carbohydrates, and fats.

is quite similar to that of all living cells. The amount of material required to meet the structural requirements of bacteria, and to replace losses incidental to the formation of soluble enzymes and other elements, is very little. Usually, also, the structural waste incidental to the elaboration of the bacterial substance is inconspicuous in amount and reactivity.

The cytoplasm of the bacterial cell is always more or less poisonous when it is liberated within the tissues of an animal or man, that of the saprophytic types of bacteria being quite as reactive on the whole in this regard as that of the very virulent organisms, as Bacillus diphtheriæ (Vaughan). The significance of bacterial infection, however, is associated primarily with the growth of bacteria in the tissues, or with the absorption into the tissues of products incidental to their growth. In other words, the energy phase of bacterial metabolism is in all probability of the greatest importance from the viewpoint of microbic infection and microbic intoxication.

The products arising from the transformation of nutritive substances into energy by bacteria are of two principal types—nitrogen-containing, or derivatives thereof, and non-nitrogenous. The former arise from proteins or protein derivatives, the latter from carbohydrates, less commonly from fats.[11]

The composition of the highly complex nitrogenous bacterial toxins, as, for example, that of the diphtheria bacillus, is unknown, although it may be separated from solution by protein precipitants, and it appears to have some points of resemblance to that group of the proteins known as the globulins. From the viewpoint of the present discussion, diphtheria toxin, and the soluble bacterial toxins in general, may be defined as soluble products of unknown but complex composition, containing nitrogen, arising from the utilization of proteins or protein derivatives for energy by specific bacteria.

In general, the measurable changes induced in the nitrogenous constituents of culture media by the great majority of pathogenic microbes, as deamination, or changes in amino nitrogen, are quantitatively the same. (See table page 677.) The nitrogenous metabolism of bacteria which produce soluble toxins, as the diphtheria, tetanus, and Shiga bacilli, is comparable in magnitude and general characteristics to that of such pathogenic bacteria as the typhoid bacillus, in whose cultures soluble, specific toxins have not been detected.

The qualitative changes induced by these same organisms upon nitrogenous [protein] substances are, on the contrary, quite unknown. The elucidation of the chemical structure of toxins and other harmful nitrogen-containing products of the transformation of protein, or protein derivatives, is a problem for the bacterio-chemist of the future to solve.

[11] There is some evidence that lecithin and similar phosphatids may be decomposed by bacterial action with the liberation of physiologically active substances.

As knowledge of bacteriology has increased, attention has been directed to the method of formation and mode of physiological action of bacterial products, derived from protein, from polypeptids, or even amino acids, other than soluble toxins. Some of these substances, as indol, are regarded by certain observers to be indicative of that condition spoken of as auto-intoxication (Combe, Bahr). Others, as betaimidazole ethylamine, possess physiological activity even in minute amounts, which may have pathological significance. Between these two general groups of substances in all probability lie the specific products of the typhoid bacillus, glanders, paratyphoid, and many others, which are perhaps neither as highly organized chemically as the soluble toxins of the diphtheria or tetanus bacilli, nor as simple as the amins derived from the aromatic amino acids.

2. General Reactions: The Formation of Phenols, Indol and Indican, Amins

The types of reactions through which proteins are transformed by bacteria into simpler compounds incidental to their utilization for energy are fairly well established, and inasmuch as certain substances of clinical importance are formed in this manner, they have a real importance in any discussion of bacterial action. It is to be remembered that each kind of organism utilizes protein or protein derivatives somewhat differently and characteristically, but in general one or more of the following reactions are involved, either successively or simultaneously in the katabolism of proteins:

1. $R.CH_2.CHNH_2.COOH + H_2 = R.CH_2.CH_2.COOH + NH_3$.
   Reductive deamination of an amino acid to a fatty acid with the same number of carbon atoms.

2. $R.CH_2.CHNH_2.COOH + H_2O = R.CH_2.CHOH.COOH + NH_3$.
   Hydrolytic deamination of amino acid to an oxyacid with the same number of carbon atoms. Lactic acid may be formed from alanin by this process.

3. $R.CH_2.CHNH_2.COOH + O = R.CH_2.CO.COOH + NH_3$.
   Deamination and simultaneous formation of an alpha ketonic acid. [Pyruvic acid transformation.]

4. $R.CH_2.CHNH_2.COOH + O_2 = R.CH_2.COOH + CO_2 + NH_3$.
   Deamination of amino acid and simultaneous oxidization, resulting in a fatty acid with one less carbon atom.

5. $R.CH_2.CH_2.COOH \longrightarrow R.CH_2.CH_3 + CO_2$.
   Carboxylic decomposition of fatty acid with the formation of a fatty acid containing one less carbon atom.

6a. $R.CH_2.CHNH_2.COOH \longrightarrow R.CH_2.CH_2NH_2 + CO_2$.

Carboxylic decomposition of amino acid with the formation of an amin,

or

6b. $R.CH_2.CHNH_2.COOH + H_2 = R.CH_2CH_2NH_2 + H.COOH$

Decarboxylation with the formation of formic acid, and an amin.

$H.COOH = CO_2 + H_2$

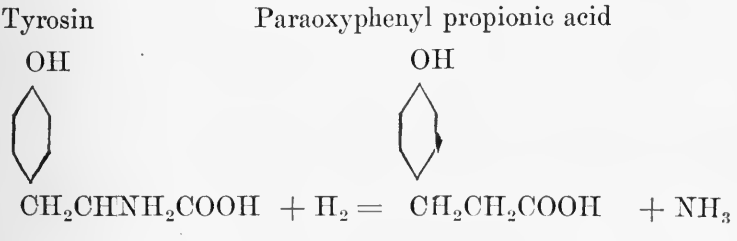
Formic acid, under the action of formiase, may be decomposed into carbon dioxid and hydrogen.

3. Reactions Illustrative of the Decomposition of Proteins by Bacteria

a. The Decomposition of Tyrosin.—Organisms like Bacillus proteus act upon proteins in solution, first by an extracellular cleavage of the protein to polypeptids, and probably peptones by the soluble proteolytic enzymes they secrete, then decomposing the polypeptids intracellularly, according to the reactions indicated. [In the alimentary canal of man, it is probable that the digestive enzymes are largely responsible for the initial cleavage of the protein molecule. The subsequent steps, giving rise to products not formed by the activity of gastro-intestinal enzymes, as indol, are the result of intracellular digestion of the protein fragments by bacteria.[12]]

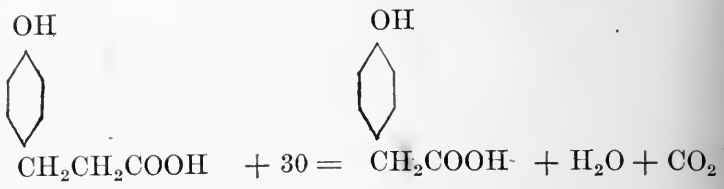
The following steps in the decomposition of tyrosin to paracresol and phenol indicate the theoretical progress of the decomposition of this amino acid to compounds, as paracresol and phenol, which have no available energy for the organism. In this state they are eliminated from the bacterial cell and appear in the culture medium, or in the alimentary canal.

1.



[12] The formation of protein-liquefying enzymes and the production of indol do not take place in cultures of Bacillus proteus containing utilizable carbohydrate.

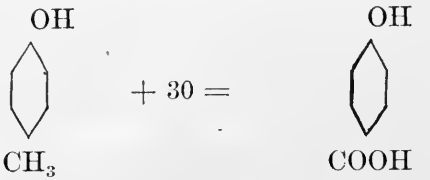
Paraoxyphenyl acetic acid

2. 

Paracresol

3. 

Paraoxybenzoic acid

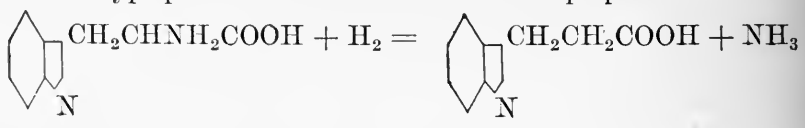
4. 

Phenol

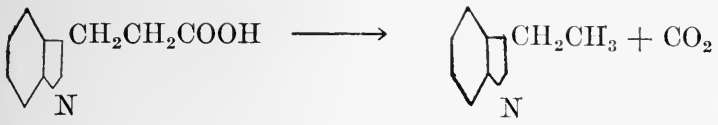
5. 

b. Tryptophan Decomposition.—Similarly, tryptophan undergoes decomposition through a variety of intermediary products, some of which, as indol acetic acid, claimed by Herter to be the urinary pigment urorosein, skatol, and indol, are of some physiological and possibly pathological significance. Bacillus coli and Bacillus proteus are the common producers of indol in the intestinal tract. [It may be repeated here that utilizable carbohydrate will prevent the formation of indol and skatol.]

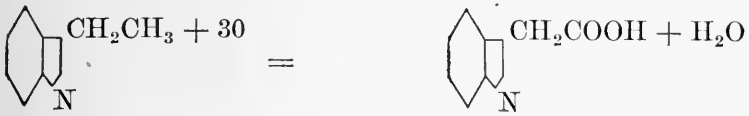
Tryptophan Indol propionic acid

1. 

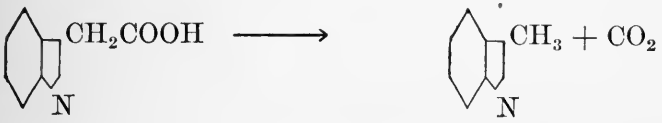
Betaethyl indol

2. 

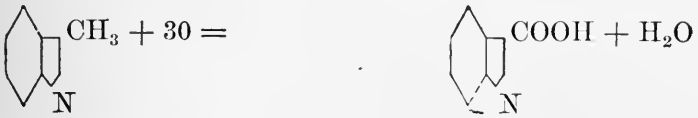
Indol acetic acid (urorosein)

3. 

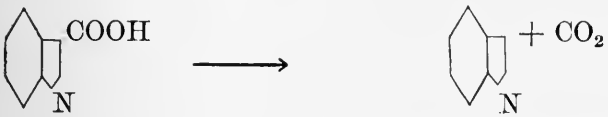
Skatol



Betaindol formic acid



Indol



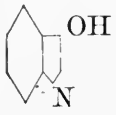
Indol is formed in the greatest amounts in those cases where intestinal putrefaction is actively taking place. Obstruction of the small intestine is a very potent factor in promoting excessive amounts (Combe). Sluggish peristalsis with the attendant relatively slow absorption of the products of protein digestion provides conditions favoring an overgrowth of Bacillus coli and other indol-forming bacteria.

Gelatin, which is deficient in tryptophan [and other aromatic amino acids] does not play a part in indicanuria. The toxicity of indol appears to be slight, and it is lessened when indol is oxidized and is paired with sulphuric acid (Herter). Amounts administered by mouth to 0.2 gram, however, appear to cause headache, malaise and lassitude.

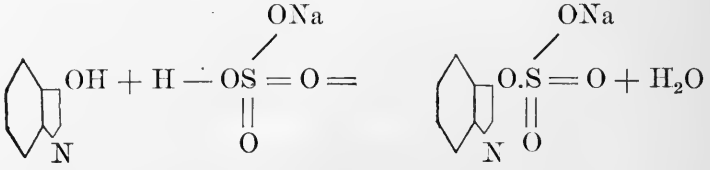
Defective oxidization in the liver may lead to a low grade indol toxemia. Herter and Wakeman found that surviving liver acts upon indol in such a manner that it cannot be recovered by distillation of the organ. The kidney and muscle are unable to fix indol in this manner.

The daily excretion of indican varies greatly, both in the period of life and with the individual. Nurslings practically never excrete indican (Soldin). Adults secrete up to 10-12 milligrams daily without symptoms (Folin and Denis).

Indol acetic acid, resulting from an oxidative deamination of tryptophan, is said by Herter to be the mother substance of the urinary pigment, urorosein. Indol is absorbed from the intestinal tract and oxidized in the body, chiefly apparently in the liver, to indoxyl:



and excreted as the sodium or potassium salt, indoxyl sodium [potassium], sulphonate, or indican. It is also excreted under certain conditions paired with glycuronic acid.



Indoxyl sodium sulphonate

Phenyl alanin undergoes decomposition similar to tyrosin, finally being absorbed from the alimentary canal and paired with glycuronic acid or with sulphuric acid. In the latter event, it becomes, together with indican, phenol and paracresol, the principal ethereal sulphates of the urine. Phenol,[13] and paracresol, resulting from the bacterial degradation of phenyl alanin and tyrosin, are excreted in considerable amounts as ethereal sulphates. Folin and Denis state that as much as 0.2 to 0.3 gram of phenol may be excreted through the urine daily by apparently normal adults. None of the substances excreted as ethereal sulphates appear to be very toxic, although long continued formation of them in the alimentary canal may be associated with severe disturbances. At the present time it may be stated that the formation of the mother substances of the urinary ethereal sulphates is an indication of bacterial decomposition of the products of gastro-intestinal digestion of proteins. This

[13] It is worthy of note that the body rids itself of phenol, cresol, and indol [products arising from the bacterial putrefaction of protein] together with sulphuric acid, which arises from the oxidization of the sulphur of protein, as non-poisonous ethereal sulphates. This combination of noxious products of protein degradation, with a minimal withdrawal of sodium or potassium would appear to be a not unimportant method of elimination of a fixed acid (sulphuric acid), without impairing to any marked degree the alkalai reserve of the body.

takes place chiefly in the small intestine. A change of diet, restricting protein and furnishing a large part of the caloric requirement above that associated with a reasonable level of nitrogen equilibrium, by carbohydrate and fat, usually will lead to a reduction of protein putrefaction through the sparing action of utilizable carbohydrate for protein in the metabolism of the intestinal bacteria.

4. The Effects of Utilizable Carbohydrate upon the Formation of Phenols, Indol and Amins

Simple decarboxylization of aromatic amino acids gives rise to amins, some of which are of significance from their physiological action. Thus, ornithin, $NH_2.CH_2.CH_2.CH_2.CHNH_2.COOH$, is changed by mixtures of bacteria acting upon protein into putrescin or tetramethylenediamin,

$$NH_2.CH_2.CH_2.CH._2CHNH_2COOH \rightarrow \begin{matrix} CH_2CH_2CH_2CH_2 \\ | \qquad\quad | \\ NH_2 \qquad NH_2 \end{matrix} + CO_2$$

and lysin similarly is decarboxylized to cadaverin:

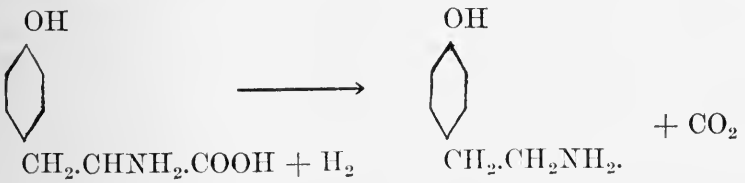
$$NH_2CH_2.CH_2.CH_2.CH_2.CHNH_2.COOH \rightarrow \begin{matrix} CH_2CH_2CH_2CH_2CH_2 \\ | \qquad\quad | \\ NH_2 \qquad NH_2 \end{matrix} + CO_2,$$

or pentamethylenediamin.

Putrescin and cadaverin were about the first of the group of substances, frequently called ptomains, to be isolated and identified. It is probable that sepsin (Fraenkel) also belongs to this class of diamins. The clinical significance of cadaverin and putrescin is not clear. These substances have been frequently detected and occasionally isolated from cases of cystinurea (Spiegel). The information available at present is insufficient to explain the relationship, however,—if, indeed, any exists.

Sepsin is said by some to be a capillary poison (Barger).

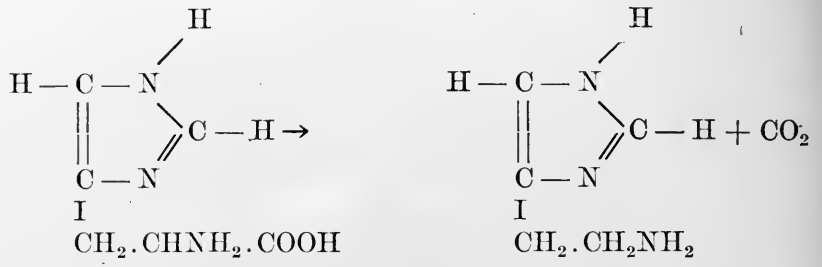
Tyrosin is changed by the loss of the carboxyl group to tyramin or paraoxyphenyl ethylamin.



Barger and Walpole have detected tyramin in meat that has been allowed to putrefy spontaneously. It appears to be a physiologically active substance that is formed in small quantities when ordinary putrefactive organisms are allowed to act upon protein in the absence of utilizable carbohydrates. Such a condition appears to be present in the alimentary tract of man not infrequently. When tyramin is injected intravenously in small amounts into dogs, it raises the blood pressure rapidly and decidedly. The same authors have shown that this substance is also an important pressor constituent in some ergot preparations.

Phenylethylamin, derived very probably from phenyl alanin, as paraoxyphenyl ethylamin is derived from tyrosin, is perhaps a pressor base, although convincing data upon this point is wanting.

Similarly, histidin, through the loss of the carboxyl group, becomes the powerfully reactive histamin, or beta imidazole ethylamin.



Ackermann has detected histamin among the products resulting from the decomposition of histidin by bacterial action. Somewhat later, Berthelot and Bertrand described their Bacillus aminophilus intestinalis, an intestinal parasite belonging to the Mucosus capsulatus group, which they believed to be the causative agent in the production of histamin in the alimentary canal. About the same time, Mellanby and Twort isolated an organism, apparently closely related to, if not identical with, Bacillus coli, which effects the same transformation. The year before, Barger and Dale had isolated histamin from the intestinal wall. Koessler and Hanke have shown recently that Bacillus coli will produce histamin from histidin in cultures of this organism.

It is significant that both Berthelot and Bertrand and Mellanby and Twort have found that the amin is not produced in acid solutions. A survey of the experiments suggests strongly that the acid which is present in such cases is derived from the fermentation of glucose. Histamin is best isolated from "putrefying" mixtures. In this connection, the observation of Garcia that glucose added to putrefying horseflesh reduces the yield of diamins very materially is significant. It would appear that utilizable carbohydrates interfere with the utilization of the protein or protein derivatives for energy, precisely as is the case with other putrefaction products described above.

Histamin is a very reactive compound. According to Vaughan, one-half milligram injected into a guinea pig will cause death very soon. The symptoms elicited suggest in a striking manner those characteristic of anaphylactic shock. There is a strong contracture of smooth muscle fiber, including that of the bronchial musculature. The latter narrows the lumen of the bronchi to a very small opening, which in connection with the somewhat tortuous course of the respiratory tract, leads to asphyxiation. There is also noticed a rapid fall of body temperature. According to the observations of Dale and Laidlaw, however, the coagulability of the blood in such cases is practically unaltered, which is a point of difference between this syndrome and that of anaphylaxis induced in a sensitized animal with the homologous protein.

It would appear from available evidence that the formation of the aromatic amins, phenyl ethylamin, paraoxyphenyl ethylamin, beta indol ethylamin, and beta imidazole ethylamin, under ordinary intestinal conditions, is chiefly the result of the activities of the colon-proteus-mucosus capsulatus group of bacilli. It is probable that these amins do not form in detectable quantities when the proportion of carbohydrate to protein of the food is sufficient, with existing alimentary conditions of absorption, to provide at least a minimal amount of sugar at the intestinal levels where these organisms ordinarily are found. A sour milk diet is supposed to restrict or prevent the formation of amins, and of other putrefactive products as well. It should be remembered that a sour milk diet is one restricted in protein, which of course reduces the amount of protein from which the parent amino acids are derived.[14] The carbohydrate content of a typical sour milk diet is decidedly increased in proportion to the protein. This furnishes a readily utilizable source of energy for the bacteria of the alimentary canal, and thereby switches their metabolism from the protein constituents. Under these conditions, lactic and acetic acids are produced largely, in place of the amins and other putrefactive products.

5. The Physiological Action of the Aromatic Amins

Generally speaking, the amins containing the benzene nucleus, phenyl ethylamin, paraoxyphenyl ethylamin, and indol ethylamin cause an increase of blood pressure upon injection, paraoxyphenylamin being the most powerful of this group. There is some theoretical ground for associating the symptoms induced in experimental animals with a direct stimulating action of the sympathetic system. Barger and Dale, in studying this relationship, have made use of the term "sympathomimetic," which seems to be appropriate.

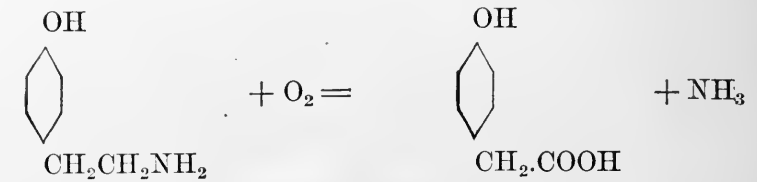
[14] Gelatin contains much less of the aromatic amino acids than the true proteins. It can not replace protein in the diet, but may be of some value for temporary dietary reduction in these compounds.

Beta imidazole ethylamin depresses the blood pressure upon injection, thus differing from the amins with benzene nuclei.

Continued formation of these aromatic amins is probably taking place within the alimentary canal in those whose diet is rich in protein, or whose peristalsis is sluggish, and in whom therefore there must be a protein residuum at levels where the colon and proteus organisms can grow. Such individuals would appear to have the bacterio-chemical basis for increased blood pressure and other symptoms indicative of the pharmacological action of these drugs. Usually such is not the case.

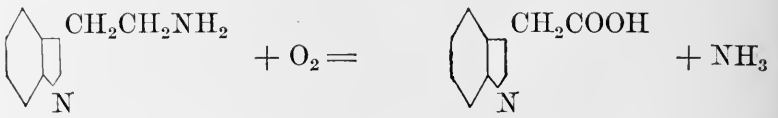
When the liver is functioning well, it appears to possess the ability of changing the aromatic amins, which are brought to it from the intestinal vessels, through a process of direct, oxidative deamination to corresponding fatty acid derivatives.

Thus, tyramin is changed to paraoxyphenyl acetic acid:



Tyramin — Paraoxyphenyl acetic acid.

and indol ethylamin is changed to indol acetic acid, thus:



Indol ethylamin — Indol acetic acid — (urorosein)

Erwins and Laidlaw have actually shown by perfusion experiments that indol ethylamin and tyramin are changed respectively to indol acetic acid and to paraoxyphenyl acetic acid. This suggests that the normal condition is one in which the amounts of aromatic amins absorbed from the intestinal contents and carried with the portal blood to the liver, are oxidized, and thus rendered adynamic in that organ.[15] Defective oxidation powers, or a flood of aromatic amins too great for the liver to handle, would lead to the escape of the unaltered amins into the general circulation, where they might well lead to increased blood pressure and associated symptoms.

The preliminary studies of Woolley and Newburgh upon the effects

[15] Folin and Denis have apparently found that the oxidization and subsequent pairing of phenols is less quantitative than had been supposed.

of injecting indol into the circulation of animals suggest that the escape of unoxidized putrefactive products, such as indol or aromatic amins, from the liver to the general circulation is more frequently a causative factor in the production of symptoms than a mere overproduction and absorption of these substances from the alimentary canal, when the liver is functioning normally.

It is conceivable, although evidence upon this point is not available, that the epithelial or underlying cells of the intestinal tract may possess to a degree the power of oxidizing or altering these aromatic amines and other putrefaction products.

Attention is directed at this point to the important studies of Simonds upon the effects of carbohydrate in liver poisoning. He says, "The administration of sugar will prove to be an important therapeutic measure in phosphorus and chloroform poisoning,—in human beings, in acute yellow atrophy and possibly in eclampsia." It would appear from his experiments and observations that inasmuch as liver enzymic activity is strengthened, even when specific poisoning has taken place, that a similar procedure would be of material benefit when the liver is permitting the escape of unoxidized putrefactive products into the general circulation. The administration of carbohydrate, it seems, is at once good physiology, good biochemistry, and good bacteriology.

Summary

Evidence has been presented that the bacterial decomposition of proteins or protein derivatives for energy may result in the production of specific, soluble toxins, aromatic, physiologically active amins, putrefactive products, such as indol or skatol, and of unknown products which are harmful in varying degrees to man. In a majority of instances, these various products, which are specific for the specific organisms, do not form in the presence of utilizable carbohydrates. In the latter event, practically all these bacteria are potentially sour milk bacteria so far as their products of growth are concerned, forming lactic and acetic acids in place of specific products of protein degradation.

Many of these protein products of bacterial formation are, or may be, found in the alimentary canal. It is obvious that a correlation may exist between alimentation, intestinal bacteria, health, and chronic or acute disease. Furthermore, the close connection between the nature of the food and the character of the products produced in the test tube may have a corresponding relationship in the human alimentary canal, inasmuch as the two reacting agents—food and microbes—are fundamentally the same in both instances. The striking parallelism between diet and bacteria is shown in the changes in intestinal bacteria which follow material changes in diet.

D. Intestinal Bacteriology

General History and Development

The earliest convincing studies of the bacteria of the alimentary canal were those of Theodore Escherich upon the intestinal flora of nurslings. This talented observer isolated and described many of the more common and important normal microbes of the intestinal tract, inventing methods for their recognition which are in use in modified form to-day. He tried to correlate their physiological processes with normal and abnormal intestinal conditions, as well. This work is of special merit, not only for its detailed information, but also for the broad viewpoint from which the work was conducted.

Comparatively little attention was paid to the work of Escherich for several years after its publication. The discovery of the cholera vibrio by Koch, in 1883, followed by that of the typhoid bacillus by Gaffky in 1884, focussed attention upon the disease-producing intestinal bacteria to the virtual exclusion of the normal organisms and their relations. Whatever progress was made in the study of the non-pathogenic types was directly associated with methods for their detection and differentiation from the pathogenic microbes. Intestinal bacteriology, in common with the entire field of microbiology, became a purely diagnostic science. This extensive interest in diagnostic intestinal bacteriology has been extremely fruitful, however. The microbes which are causative agents in practically all the acute intestinal infections of exogenous origin are now well known, and the domain of preventive medicine has profited greatly through the accumulated information relating to the cycles of infection of these bacteria.

Escherich was unable to isolate the predominating organisms of the normal nursling feces, although he recognized them morphologically and realized that he was unsuccessful in this direction. It remained for Tissier to accomplish this difficult task, and with his studies of Bacillus bifidus communis, the way was cleared for satisfactory studies of the intestinal bacteria from birth to adult life.

The discovery of paratyphoid bacilli by Salmon and Smith, Gärtner, and Brion and Kayser, and their significance by Achard and Bensaude, and of the dysentery bacilli by Shiga and Flexner, practically completed the list of bacilli which induce extensive epidemic intestinal disease in man.

Attention was then of necessity directed to the endogenous intestinal organisms. Advances were made in two principal directions—the isolation of bacteria from the normal intestinal contents and their identification, and, secondly, the study of intestinal microbes at different periods of

life. The former studies, which culminated in the comprehensive monograph by Ford, showed quite clearly that the normal organisms were quite closely related to the coli, proteus and mesentericus groups. This is suggestive in that the normal bacilli of the alimentary canal which exhibit chemical characteristics common to the colon-proteus-mesentericus types remain dominant throughout adult life.[16] Observations by the author upon the residual intestinal flora of a man who starved for thirty-one days supports this view.

The other line of study considered more specifically the relations which exist between the normal or abnormal chemical peculiarities of intestinal processes of microbic causation, and the activities of specific bacteria. The comprehensive monograph of Herter, summarizing his extensive contributions to the field of excessive bacterial activity in the alimentary canal, epitomizes the information upon this phase of the subject. Herter also clearly recognized that the injection of lactic acid bacilli into the small intestine of dogs reduced the excretion of ethereal sulphates in the urine, while Bacillus coli and Bacillus proteus appeared to increase intestinal putrefaction, thus foreshadowing the "lactic acid therapy" which Metchnikoff so forcefully presented in his work upon the prolongation of life. About this time Sittler studied and summarized the corresponding information with respect to the nursling.

During this period of approximately twenty-five years there was an ever-increasing precision of methods, both chemical and bacteriological, and the last decade has witnessed the application of these procedures to the study of bacterial metabolism under various conditions. As a result of the application of these more refined methods to the study of bacteriological activities, a new viewpoint has presented itself. Many of the conflicting statements and observations which had embarrassed earlier investigators have been reconciled, and a fairly definite unification of the phenomena underlying bacterial chemistry has led to renewed interest in the highly important field of bacteriotherapy.

Some of the more important relations of bacteriochemistry to bacterial metabolism in the alimentary canal follow.

1. The Intestinal Bacteria of Normal Nurslings

The Relation Between Diet and Microbic Response.—The entire alimentary canal of the newly born babe is sterile under normal conditions, and the first bacteria appear in the intestinal tract several hours after birth (Escherich). This earliest infection of the alimentary canal is by adventitious organisms derived from the environment of the infant. The kinds of microbes found at this time are those which have gained en-

[16] This applies only to adults. The flora of nurslings is quite different and distinct with reference to the type of bacteria and their characteristics.

trance through the mouth to the alimentary canal from various sources, and their numbers—up to the third day of life—are determined chiefly by their ability to grow in the fetal intestinal detritus, and the cholostrum. In temperate zones, the initial microbic growth is usually more luxuriant in summer than in winter.

On or about the third day after birth, the nature and appearance of the alimentary microbic flora undergoes a clearly discernible change (Tissier). The variety of forms and dissimilarity of staining reactions which characterize the postfetal flora give way to the dominance of a rather long, slender bacillus with slightly tapered ends which rapidly supplants the adventitious types. This is Bacillus bifidus (Tissier), a lactic-acid-producing bacterium, characteristic of the intestinal and fecal floras of a great majority of normal nurslings. It is worthy of comment that Bacillus bifidus becomes prominent synchronously with the full flow of the breast milk. Breast milk, it will be remembered, contains more than six per cent of lactose, and scarcely one and a half per cent of protein. In addition to Bacillus bifidus, other bacteria in much smaller numbers are found normally,—Micrococcus ovalis, Bacillus acidophilus, and even fewer members of the colon and lactis aërogenes groups [the feces stained by Gram's at this time are strongly positive]. The author has found that these organisms without exception can grow extremely well in mediums rich in lactose, and they all produce considerable amounts of lactic acid. The combined acidity arising from the utilization of lactose for energy by these bacteria is the principal source of the acid reaction characteristic of the normal intestinal contents and feces of the nursling. Lactic acid, in the concentration normally present in the intestinal tract, restrains the growth of endogenous proteolytic bacteria, and it also restricts the development of exogenous, pathogenic microbes which gain entrance to the tissues through the alimentary canal.[17]

When, for any cause, as for example decreased peristalsis, the lactose is absorbed in the higher levels of the tract, a purely protein residuum is left in the lower levels of the small intestine, and in the large intestine. Under these conditions, the habitat of the obligate acidogenic bacteria is restricted, and they are greatly reduced in number and in activity. This follows through their inability to grow well in a residuum in which protein derivatives are their only source of energy.

The immediate effect is a greater or lesser reduction in the amount of lactic acid[18] formed in the intestines, and in consequence of this

[17] In this connection, the observations of the Medical Research Committee that dysentery bacilli may be isolated from dejections having a neutral or slightly alkaline reaction, for days after they are excreted, are of interest. It was found that dysentery bacilli could not be isolated from the same stools having an artificially induced acid reaction (lactic acid), approximately that of the normal nursling movement, even after a few hours.

[18] All the lactic acid bacilli appear to produce some acetic and formic acid together with minute amounts of similar volatile decomposition products of the fermentation of

reduction the principal obstruction to the development of endogenous proteolytic bacteria, as Bacillus proteus and Bacillus mesentericus, is removed, or at least greatly reduced. Also, the absence of lactose and other utilizable carbohydrate at the level of the tract where Bacillus coli and related forms are most numerous forces these organisms to become proteolytic in place of fermentative. The net result is an immediate increase in proteolytic activity, and a decided extension of the proteolytic zone.

Indol and other decomposition products resulting from the utilization of protein for energy are formed in increasing amounts from the intestinal contents, and these may be absorbed from the tract and excreted as aromatic sulphates or glycuronates into the urine. Peristalsis may be, and frequently is, further reduced by this process, which tends to become therefore of the magnitude of a vicious cycle.

The biological basis for successful invasion of the intestinal tissues by exogenous microbes is probably created or at least augmented hereby, because available evidence indicates that intestinal invasion is more readily accomplished when the proteolytic activities of bacteria exceed, or replace, the normal fermentative processes.[19]

Bacteriologically considered, therefore, the normal nursling intestinal flora reacts with breast milk in the alimentary canal in a manner analogous to the natural souring of milk outside the body. Both are essentially preservative processes. Milk soured by lactic acid bacilli does not readily undergo putrefactive changes which render it unfit for human consumption. Similarly, the normal intestinal contents of the normal nursling do not appear to undergo putrefaction.

The lactic acid, representing some decomposition of lactose, has fuel value for the body; hence, it is not an entire loss in terms of the original caloric value of the milk. In this respect, it is in sharp contrast with the products arising from the degradation of proteins of milk by bacteria which do not ferment lactose. Such putrefactive products as are known are either useless, or more or less harmful to the human body when absorbed from the alimentary canal.

It would appear therefore that a natural relationship exists between the nature of the diet of the nursling and the character of the products formed in the intestinal tract which are qualitatively those formed in the natural or artificially induced souring of milk outside of the body. The bacteria concerned are chemically, but not specifically, the same. Intestinal conditions are unlike those outside of the body. This is true not

the lactose, and to a much lesser degree from fats:—for convenience, the lactic acid will be mentioned as the principal product, and indicative of the entire group of acidic compounds.

[19] The theoretical advantage of preparing patients for surgical operations, especially those upon the large intestines, by the induction of a suitable fermentative flora in place of a putrefactive flora is suggested. Of course this applies to operations which are not emergency cases, since time is required to effect this change.

only with respect to temperature [that of the body being 37.5° C., and that of the outside world varying with climate and season], but also in association with those purely intestinal factors of secretions, including bile, enzymes and products of enzyme activity. These ancillary factors exercise a not immaterial influence upon prospective intestinal tenants. It is significant, however, that notwithstanding these environmental differences, the intestinal souring of milk is the qualitative equivalent of the spontaneous souring outside of the human body. The significant factor is the continuous availability of lactose in both processes.

Experimental Evidence of the Effects of Sugars upon the Intestinal Flora.—Many studies upon experimental animals have shown the effects of utilizable carbohydrates, as lactose, glucose, and other bioses, and polysaccharids, upon the establishment of an intestinal flora in adult animals and man. When such substances are added to the diet in sufficient amounts to permeate the entire absorptive length of the alimentary canal, the flora induced is the chemical replica of that of the normal nursling. When the carbohydrates are reduced or eliminated from the regimen, proteolytic bacteria rapidly gain the ascendency.

Escherich appears to have been the first observer actually to perform dietary experiments upon animals. Dogs were selected. A four weeks' old puppy was fed first upon milk, then upon meat. The changes in the character of the excreta and of the bacteria in the excreta were observed in each instance. A milk diet led to the evacuation of bright yellowish dejecta, the consistency and odor of which were reminiscent of those characteristic of the normal nursling. The organisms detectable were very similar to those of a normal nursling.[20] Gelatin-liquefying bacteria were few in numbers, but coccal forms became more numerous.

The substitution of meat for milk induced a striking change in the appearance of the feces, and in the character of the fecal bacteria. The former lost their golden yellow color and became dark in color, smaller in bulk, and possessed of a fecal odor, suggesting in this respect that of a normal adult. Gelatin-liquefying bacteria increased very decidedly in numbers and in activity. Coccal forms were relatively diminished. Spores of proteolytic organisms, presumably of the mesentericus group, became prominent in stained smears from the meat-diet feces, and the entire picture, bacterial and chemical, so far as determinations were possible, suggested that the entire intestinal condition induced was similar to that of normal adults.

Following this monumental work of Escherich, which was so carefully carried out but unfortunately limited because of the meager fund of bacterial knowledge and the lack of adequate chemical methods avail-

[20] It should be remembered that the dominant organism of the typical nursling's feces—Bacillus bifidus—was not known in Escherich's time. It was isolated nearly fifteen years later (Tissier).

able at that time [1886], a series of investigations appeared which added many detached facts to the problem of intestinal bacteriology.

The discovery of the dysentery bacillus in 1898, and of Bacillus bifidus in 1900, marks the close of the older period of the study of intestinal bacteria. The greatly improved cultural methods, both aërobic and anaërobic, which resulted in the isolation and identification of closely related types of organisms, as the several types of dysentery bacilli, focused attention upon the value of carbohydrates, or derivatives of carbohydrates, for diagnostic purposes in bacteriology. The decade between 1895 and 1905 was particularly noteworthy for the numbers of new types and kinds of bacteria, both aërobic and anaërobic, which were detected by this procedure.

The problem of the intestinal bacteria was restudied, by the author, with the great advantage of reasonably accurate methods of bacterial and chemical procedures in 1909. The relationship between diet and intestinal flora was observed, and the general phenomena relating to the alternations in dominance of fermentative and putrefactive intestinal floras in response to carbohydrate and protein regimens were elucidated at this time. The first observations were made upon cats and monkeys. It was found that both carnivorous and omnivorous animals responded to the same dietary changes in a similar manner.

The striking features were the dominance of an acidogenic intestinal flora, similar to that of a nursling, upon a carbohydrate diet [glucose added to milk], and the dominance of proteolytic bacteria in the alimentary canal upon a purely protein diet. The urinary changes also were significant. Upon a carbohydrate regimen the urinary products of putrefaction, as indican and phenols, were greatly diminished, or absent. This corresponded to the chemical activities of the nursling bacteria cultivated outside the body. Such organisms do not form indol or phenol in culture media. The return to a protein diet was followed very soon by the appearance, or great increase, of the indolic and phenolic substances of the urine. The fecal bacteria from such diets were predominantly proteolytic and reproduced in culture medias under proper conditions the antecedent substances from which indican and the ethereal sulphates are derived.

It would appear from these observations that there was a very definite and controllable relationship between certain diets, the bacterial types of intestinal flora, and the presence or absence of urinary putrefactive products. These experiments were repeated, greatly amplified, and confirmed in a later series (Herter and Kendall).

The following observers, Bahrdt and Beifeld, Sittler, Rettger and Horton, Torrey, Hartje and Klotz, have since corroborated the principle of the alternation of bacterial types in the alimentary canal in response to definite dietary stimuli, and have extended the field by indicating the

selective effects of various carbohydrates upon the types of lactic acid producing microbes which become dominant in the intestinal tract as one or another sugar is added to the diet.

A more recent series of observations by Torrey has not only amplified this particular aspect of the subject and confirmed anew the principle of the bacterial response to dietary alternations, it has also shown that fats play a very minor, or entirely negligible, part in this process.

In general, therefore, it may be stated that the normal nursling intestinal flora is essentially fermentative in character. It represents the natural bacterial response to a definite nutritive condition created within the alimentary canal by the continuous passage of milk sugar—lactose—throughout the absorptive area. Furthermore, it is possible to reproduce essentially the same chemical activities and bacterial types in the intestinal tracts of experimental animals, both carnivora and omnivora, by the administration of the diet of the normal nursling.

2. Adolescent and Adult Intestinal Bacteriology

Adolescents and adults, unlike nurslings, are normally omnivorous. The proportions of proteins and carbohydrates [principally starches and dextrins] in the average adolescent and adult diet are more nearly equal than is the case with nurslings or milk-fed children. The large intestine, from the cecum to the rectum, therefore, becomes more and more a receptaculum of the products of protein digestion, and of protein derivatives altered by bacterial digestion. The tendency is for putrefactive processes to predominate, due to the more or less periodic intervals of carbohydrate disappearance. These periods of carbohydrate presence and absence exercise a very decided influence upon the types of bacteria which can thrive under these intervals of carbohydrate and protein offerings for energy. The obligate lactic acid flora, either Bacillus bifidus or Bacillus acidophilus, according to Moro, Finkelstein, and the author, dies out and the succeeding bacteria are of the colon type, which, as has been stated before, can utilize protein for energy nearly as well as carbohydrates.

Organisms of the Bacillus coli type, in fact, are the dominant bacteria of the intestinal and fecal flora in normal adolescents and adult life, when the ordinary mixed diet is that of the dweller of the temperate zone. Under such conditions some indol is formed in the alimentary tract and in many individuals at least—more frequently those who are heavy protein eaters—it will be found as indican in moderate amounts in the urine.

The conditions under which indol is formed are also favorable to the formation of aromatic amins, as histamin, indol ethylamin, or even

tyramin. The bacteria which can form amins by the decarboxylization of the aromatic amins are not thoroughly studied. Berthelot and Bertrand have described Bacillus aminophilus, a member of the Mucosus capsulatus group, but according to Koessler and Hanke, Harai, Yoshimura, Guggenheim, Einis, and Berthelot, it is probable that a number of intestinal bacteria can decarboxylize these compounds.

The amounts of the putrefactive derivatives of the aromatic amino acids found in the urine of normal adults under normal dietary conditions are not large in proportion to the amount of protein ingested. The figures for indican and phenolic bodies, chiefly phenol and paracresol, are the best known because these substances give color reactions which are quantitative, or approximately so; consequently, fairly accurate measurements are possible. About 10 milligrams of indican and about 0.3 gram phenolic bodies are usually found (Folin and Denis). The fecal content of indol and phenols under these conditions is unknown, although a variable amount of each must escape absorption.

At times, particularly in purulent infections incited by Staphylococci, and to a lesser extent by Bacillus coli and Bacillus proteus, some indican may properly be of parenteral origin, it being well known that these organisms form indol and phenols from the degradation of tissue and blood proteins. This is not the usual source of the urinary putrefaction products, however; as a rule they are derived solely from bacterial activity in the intestinal tract.

Obstruction of the lower levels of the small intestine, intestinal stasis, and, in general, any factor which leads to an upward extension of the habitat of Bacillus coli and related forms, is a potent factor for increased protein putrefaction.

It should be noted that the relative desiccation of the intestinal contents at the lower levels of the large intestine, together with the accumulation of products of bacterial proliferation carried down from higher levels, restricts materially the intensity of growth and activity of the intestinal flora from the transverse colon to the rectum. On the other hand, the relative emptiness of the upper small intestine, particularly the duodenum, in interdigestive periods, has been emphasized by Escherich, Tissier, and the author and is correlated with a periodic diminution of bacteria, most of which are carried downward mechanically with the food. The net result is a large fluctuation in the numbers of bacteria in the duodenum, corresponding approximately with the ebb and flow of the duodenal content of food, and a gradual increase in numbers and decrease in fluctuation, as the ileum is reached, where an intestinal residuum is almost constantly present.

At the rectum, the number of living microbes is very greatly reduced, although the corpses of bacteria [which appear to be insoluble in the digestive juices] are present in enormous numbers. It has been estimated

that fully eighty per cent of the bacteria seen in the feces are dead or so weakened in vitality that they can no longer be cultivated in artificial mediums. In other words, the most intense bacterial proliferation is in the lower ileum, the cecum, and the ascending colon.

The types of bacteria vary at the different levels. In the duodenum and jejunum, where the carbohydrates are ordinarily abundant during digestive periods, the amylolytic bacteria—those which thrive best where starches are present—are found in dominating numbers.[21] At the lower levels, facultative bacteria, as Bacillus coli—which can grow well upon a carbohydrate or upon a protein diet—are found to be the principal types. The carbohydrophilic bacteria are carried to these levels with the downward passage of the intestinal contents, but gradually decrease in numbers as well as activity with the diminution of the sugar content of the intestinal medium.

In the cecum a considerable number of types of bacteria are found, chiefly those which thrive upon a protein regimen. Starches appear to play a minor part in determining bacterial types, especially in the lower levels of the alimentary canal; the products of hydrolysis of the ordinary starches are glucose, and polymers of glucose. These are not liberated in considerable amounts at any one time, and the soluble products of hydrolysis are usually absorbed relatively rapidly. Under these conditions the effect of starches upon intestinal bacterial metabolism, particularly with reference to their sparing action for protein, is not great. The observation of Torrey is that fats do not apparently play a prominent part in the nutrition of intestinal microbes.

It is not difficult to advance an explanation of the sudden rise in indican when an intestinal obstruction is created. In such cases, carbohydrate is removed more rapidly from the intestinal contents than the protein, leaving a nitrogenous pabulum for the bacteria. The gradual filling of the intestines to the higher levels encourages a corresponding extension upward of the habitat of the indol-forming bacteria of the colon type and the periodic emptying of the duodenum no longer is a factor in sweeping down the organisms which are resident there. The net result is an upward extension of the putrefactive flora, and an aug-

[21] Surgical operations involving the small intestine are said to be less frequently complicated by bacterial infection than those of the large intestine. The suggestion is offered that the microbes of the upper small intestine are not only fewer in numbers but are also lactic acid producing, and therefore fermentative rather than toxicogenic in their activities. Whatever of carbohydrate (starch or sugar) there may be in the food is absorbed chiefly from the intestines—not from the stomach (Howell)—and therefore the upper levels are periodically or even constantly bathed in this group of non-nitrogenous substances. In the interdigestive periods the food passes downward, carrying a majority of the bacteria with it. This appears to be an explanation of the prominence of acidogenic bacteria in the duodenum.

At the lower levels, the normal adult intestinal flora is facultative with reference to proteolysis; such organisms are more commonly found to be incitants of infection than the more strictly or obligately acidogenic forms.

mentation of its activity beyond normal. Indol and other substances are formed in increased amounts and, for a time at least, appear to be absorbed from the intestinal contents [which are not desiccated at these levels] into the blood stream. Very shortly thereafter the normal capacity of the liver to oxidize the indol to indoxyl, and to pair the latter with sulphuric acid [or, more accurately, with the monopotassium salt of sulphuric acid] is exceeded, and there is an overflow of indol into the general circulation.

Normally, the indol and phenols, and other products arising from the bacterial decomposition of aromatic amino acids, are oxidized in the liver, as indicated in a preceding article, before they enter the general circulation. They are excreted from the circulation chiefly as aromatic sulphates, but whenever the available sulphate is decreased in amount, the body produces glycuronic acid, and pairs these aromatic nuclei with that substance prior to elimination through the kidneys into the urine. By this process the body is rid of these somewhat toxic putrefactive substances, their toxicity being reduced materially by the dual process of oxidization and pairing with sulphuric or glycuronic acid.

The phenomena of intoxication ordinarily ascribed to indol, and probably participated in by other aromatic residues of amino acids, are frequently associated with one or more of three factors; first, the continued production of unusual amounts of indol formed in the alimentary canal as the result of an unsuitable amount of protein in the diet, or persistent intestinal stasis, or both. This may lead to the absorption of amounts of the aromatic nucleus beyond the normal capacity of the liver, and the excess of indol then may appear as such in the general circulation. Secondly, defective oxidative power of the liver, leading again to the systemic flooding with indol; or, finally, an impaired power of combining the oxidized indol with sulphuric or glycuronic acid.

Any of these processes, imperfectly carried out, may result in the slow, cumulative effects which eventually are recognized clinically by lassitude, malaise, headache, and dizziness, and other symptoms spoken of as "auto-intoxication."

It is quite as possible for an individual to suffer from an excessive production of lactic acid of intestinal origin as it is to be injured by an overproduction of indol or other bacterial derivatives of the aromatic amino acids. Such conditions have been described by Escherich, Finkelstein and Salge. The few cases on record occurred in young children, once in almost epidemic proportions, in a hospital in Gratz.

The causative factor appears to be an upward extension of the normal zone of growth of Bacillus acidophilus, or a closely related organism, into the small intestine. The most prominent symptom is a profuse, watery diarrhea. The dejections are yellowish and have a very sour smell. The acidity in the few cases studied was found to be four to eight or even

ten times that characteristic of the normal acidophilic stool. In spite of the great prostration, there was little evidence of a toxemia of alimentary origin. The removal of all carbohydrate from the diet appeared to reduce the excessive acidity quite promptly. Excessive lactic acid production in the digestive tract is uncommon.

3. Sour Milk Therapy and Bacterial Metabolism

For more than two decades, evidence relating to possible correlations between products of protein putrefaction in the alimentary canal and those somewhat general symptoms designated by many observers "auto-intoxication," has been collecting. Metchnikoff, following a suggestion by Herter, wove the various observations and facts upon this subject into a coherent theory covering the salient features and advanced his sour milk therapy as a remedial procedure to combat these conditions.

Briefly, the Metchnikoff hypothesis is as follows: In advanced adult life, or earlier, the intestines become populated with bacteria, chiefly anaërobic, which produce indol and other putrefactive products in unusual or intolerable amounts. The antecedent cause is a protein-rich diet. The absorption of these substances for variable periods of time leads to arterial hardening and that series of structural changes which is frequently spoken of as premature senility. The site of trouble, says Metchnikoff, is chiefly the large intestine. In support of this view, two or three instances are cited in his book in which patients suffering from so-called intestinal toxemia were benefited by the shortening or removal of the large intestine by surgical operation. By so doing, the offending bacteria and their environment were simultaneously eliminated.

In contrast to this possibility, that longevity and the normal approach to uncomplicated old age are interfered with to a degree by excessive bacterial putrefaction in the cecal cesspool, attention was directed to the unusual span of life enjoyed by some of the Biblical patriarchs (Piffard). Metchnikoff also found that longevity is, or was, a noteworthy characteristic of those inhabitants of southeastern Europe who drink milk soured by lactic acid bacteria as a principal article of food.[22]

The suggested relationships between soured milk,[23] sour milk bacteria, longevity, on the one hand, and mixed diets, intestinal putrefaction and auto-intoxication, with premature senility on the other hand, have led Metchnikoff to conceive of the possibility of replacing the putrefactive intestinal flora by the lactic acid bacilli of Bulgaria. Replacing malig-

[22] Souring is induced by adding to the freshly drawn milk lumps of coagulated casein containing impure cultures of lactic acid bacilli, known variously as Kephir granules, Lebenraib, Maadzoun, Yoghourt, and by other names.

[23] The souring of milk is the only method of preservation in warm countries where refrigeration can not be practiced.

nant microbes by beneficent bacilli, and encouraging the latter to colonize in the large intestines as a safeguard against future endogenous poisoning, is the essence of the Metchnikoff hypothesis.

The method of administration of the Bulgarian sour milk bacillus was through milk which first was to be sterilized, then inoculated with a pure culture of the organism, and set aside to ferment to a high degree of acidity. Milk thus soured and populated with enormous numbers of Bulgarian bacilli was to be drunk in large amounts daily. It will be seen that the objective to be attained was to introduce naturally preserved milk [soured milk] containing preformed lactic acid, into the alimentary canal, in the expectation that it would not undergo putrefaction there. Also, that the Bulgarian bacillus would become resident, and supplant the native putrefactive microbes.

The results have, on the whole, been disappointing from the clinical point of view, although sour milk has unquestionably become a popular beverage. It is unfortunate that the emphasis was laid upon the acclimatization of the bacilli of Bulgarian kephir granules in the alimentary tract of man. Available evidence through the work of Herter and Kendall, and Rahe, indicates they do not grow in the alimentary tract in competition with the normal intestinal flora. From à priori considerations there is little justification for the belief that they would grow there. Observations upon the alimentary flora of normal or milk-fed nurslings have never revealed the presence of Bulgarian bacilli. It might confidently be expected that lactic acid producing bacteria, parasitic in milk, would grow if they could endure the intestinal environment. On the contrary, the human intestinal lactic acid bacilli which thrive on a milk diet are Bacillus bifidus in the normal nursling, and Bacillus acidophilus in artificially fed babies.

One of the important details of the Metchnikoff sour milk therapy procedure is a restriction of the protein in the diet of the patient. It is quite clear that rigorous attention to this factor is of unqualified benefit. To make up the requisite caloric [energy] content of the food, some sort of carbohydrate is recommended. It was surmised that the carbohydrate might also help establish the Bulgarian bacillus as an intestinal inhabitant.

It may be stated that the chief value of the sour milk therapy as outlined above was to introduce considerable amounts of preformed lactic acid. There appears to be little doubt that this lactic acid of exogenous origin is an important restrictor of certain types of intestinal fermentation, especially that in which the "gas bacillus" is either a causative factor or at least an indicator through its unusual luxuriance of growth (Kendall and Smith, Hewes and Kendall, and Simonds).

There is no very definite proof that anaërobic bacteria are important factors in intestinal putrefaction. Indeed, the evidence points to Bacillus

coli and related forms as the more common organisms which produce indol in the alimentary canal.

From what has been stated above, the increase in carbohydrate and a restriction of the protein in the diet tend of themselves to change the nature of the products formed by colon and other bacilli from the indolic to the lactic type. If enough carbohydrate can be ingested to maintain a carbohydrate content throughout that portion of the tract where bacterial proteolysis is dominant, the substitution of lactic acid for products of protein putrefaction through the shifting of the metabolism of the facultative bacteria, as Bacillus coli, naturally follows. The success of the dietary change will depend in no small degree upon the extent to which carbohydrate may be kept *continuously* in the alimentary canal. In general, therefore, it may be stated that the chief beneficial results observed in cases of so-called intestinal auto-intoxication which have been dieted upon Bulgarian lactic acid milk are to be ascribed largely to the restriction of the protein, and to an increase in the carbohydrate.

This leads to a diminution of the protein residuum in the intestine, to the shifting of the metabolism of the intestinal putrefactive bacteria, and to lactic acid production in place of indologenesis. The increase of peristalsis, and partial or complete relief from constipation, which not infrequently follows the change from a basic to an acidic reaction in the middle segment of the alimentary tract, may also be a factor in the beneficial process.

Since the publication of Metchnikoff's work, many attempts have been made to secure cultures of lactic acid bacilli for purposes of lactic acid implantation. None of these to date are selected with a view to their fitness for intestinal acclimatization. The efforts have been to seek for milk parasites, which will produce a smooth, palatable and very acid sour milk outside the human body. Some cultures have even been dispensed as tablets or lozenges. The bacteria in such preparations are dried, much like commercial yeast cakes, and are to be taken in this form. Frequently, the directions for using these dried cultures of bacteria fail to indicate that sugar be taken with the bacterial tablets. It must be obvious that these bacteria, or almost any other bacteria, cannot be expected to produce therapeutic amounts of lactic acid unless they are provided with a source of energy from which lactic acid may be formed.

If, therefore, intestinal implantation of normal lactic acid bacilli is to be practiced, it would appear logical to select normal intestinal lactic acid bacilli for inoculation into milk, intended for therapeutic purposes, or for ingestion as pure cultures, and to maintain these cultures under conditions which shall guarantee they have not lost their intestinal parasitism in favor of parasitism upon artificial media outside the body (Rotch and Kendall). It is not improbable that frequent passage of such cultures through the alimentary canal will be found essential to maintain

their intestinal parasitism, quite as frequent passages of pneumococci through experimental animals are required to maintain their virulence.

To summarize: there appears to be an abnormal state or condition, more common in adults of middle age or older, in which available evidence points to putrefactive products, the results of bacterial decomposition of protein residues in the alimentary tract, as the underlying cause. This state or condition is referred to by many as "auto-intoxication."

If such be the case, the cure, or at least the arrest, of the morbid process, naturally would be a restriction or prevention of the putrefactive bacterial processes within the alimentary canal. The bacteria which are known to produce indol, aromatic amins, and other similar putrefaction products associated with the phenomena of auto-intoxication are for the most part microbes of the colon-proteus-mesentericus groups. These bacteria produce the putrefaction products when they utilize protein or protein derivatives for energy. When they utilize carbohydrate for energy, these same bacteria produce lactic and other acids. If periods of ebb and flow of carbohydrate occur in the alimentary canal, where these organisms are abundant, there will be corresponding alternate periods of putrefaction and fermentation.

It follows that a continuous supply of the proper kind of carbohydrate will result in a continuous production of lactic acid. Implantation with normal intestinal lactic acid bacilli, as Bacillus acidophilus, with a continuous supply of carbohydrate, will tend theoretically at least to diminish the numbers of colon-proteus-mesentericus types, and restrict their activities. Such a procedure probably will be found to be feasible in a proportion of appropriate cases.[24]

Lactic acid or sour milk therapy has not yet reached its final development. The brilliant conception of its possibilities as a contribution to gastro-intestinal therapy is a monument to Metchnikoff's genius and constructive imagination.

The discussion of intestinal bacteriology thus far has revealed two distinct but related types of response to dietary alternations: First, a change in the type of bacteria, as, for example, the dominance of Bacillus bifidus in the normal breast-fed infant, and, secondly, the change in metabolism as protein or carbohydrate is available for the energy requirements of the bacteria. The dominance of types is usually met with when the diet is monotonous, and with a preponderance of one or another type of energy-producing substance. In the case of milk in the normal nursling, the seven per cent of lactose is the determining factor. On the other hand, when the energy producing substance changes from time to time, as for example in the lower levels of the small intestine of adults, where periods of carbohydrate ebb and flow are superimposed upon a protein

[24] Certain ill effects of unrestricted feeding of carbohydrate are discussed under Endogenous Intestinal Infections, *vide infra*.

residuum, bacteria which are accommodative to alternations in metabolism are confidently to be looked for. Such happens in the adult alimentary canal, and facultative bacteria, as Bacillus coli, which can accommodate their metabolism to protein or carbohydrate energy, become the dominant organisms.

The nature and extent of bacterial acclimatization in the intestinal tract is not a matter of indifference to the host; the character of the normal resident flora is of equal or greater importance.

It is conservatively estimated that a normal, healthy adult, enjoying an average mixed diet, excretes daily in the feces from one hundred to thirty hundred billion of bacteria (Schmidt and Strasburger, McNeal, Latzer and Kerr, and Cammidge). The dried weight of this bacterial mass would exceed five grams, and the nitrogen in it alone would weigh nearly seven-tenths of a gram. It is apparent that the ingested food does not contain this prodigious number of bacteria, and, furthermore, the kinds of organisms isolatable from the excreta do not coincide in type or proportion with those of the regimen. Indeed, many of the latter do not appear to endure intestinal conditions and the bacterial antagonisms therein. It must be conceded, therefore, that the alimentary canal is a singularly efficient incubator and culture medium from the bacterial point of view; an environment in which bacterial growth along rather definite lines exceeds in intensity and selectiveness that of any known natural process.

The range of reaction and the composition of nutritive substances at different levels are such that theoretically a great variety of organisms, capable of growing at body temperature, might find conditions favorable for their development. Notwithstanding the nutritive possibilities throughout the alimentary canal, from starches to glucose and fermentation acids, from practically unaltered protein to amino acids and extractives, and from fats to fatty acids and glycerin, the number of types of bacteria which occur normally and in significant numbers in this incubator-culture medium is surprisingly small. They are also fairly well known.[25]

The underlying principles of normal intestinal bacteriology, in the light of available information, may be summarized from the clinical viewpoint as follows:

1. The constant temperature, variety of food, and range of reaction in the alimentary canal create conditions favorable to bacterial growth.

2. The bacterial response to these conditions is enormous, viewed

[25] A distinction is made between the resident bacterial types which persist under normal dietary conditions for considerable periods of time, and those transient forms which successfully run the intestinal gauntlet, and which may be encountered in any massive bacterial process. Exogenous pathogenic bacteria, which will be discussed below, are specifically excluded from the present discussion.

from the standpoint of numbers—a normal adult eliminates daily several hundreds of billions of microörganisms in the feces.

3. The opportunities for bacteria of the most varied kinds to enter the mouth and to pass to the intestinal tract are almost unlimited. At one time or another virtually all bacteria from the outside world may thus become prospective tenants. Notwithstanding this possibility of a most varied immigrant flora, the predominant and, presumably therefore, the normal intestinal flora is composed of strikingly few types. The daily proliferation of these few types is responsible for the bulk of bacteria excreted in the feces.

4. Starvation reduces the number of bacteria materially, but the types found in the intestinal flora under such a condition are of the normal kinds.

5. A monotonous diet, in which carbohydrate continuously permeates the intestinal tract, leads to a simplification of the intestinal flora. In normal nurslings, obligately acidogenic bacteria of the bifidus type become dominant. In dextrin-starch mediums, members of the Bacillus acidophilus type predominate.

6. The products characteristic of the activity of the obligate fermentative flora are normally innocuous and in a measure protective, in that the lactic acid generated is a deterrent to the growth of non-fermentative [putrefactive] organisms. A similar phenomenon is observed in milk soured outside the body. It does not ordinarily putrefy.

7. It is sometimes observed that an overgrowth of acidogenic bacteria, as Bacillus acidophilus, may lead to intestinal disturbances, particularly in young children. An overgrowth of the gas bacillus [Bacillus welchii] may also lead to, or be associated with, severe intestinal disturbances which may become serious.

8. Upon a diet in which the proportion of carbohydrate to protein is nearly equal, leading to periods of ebb and flow of carbohydrate in the lower levels of the intestinal tract, the facultative organisms, members of the colon-proteus-mesentericus groups, become the principal kinds met with. Such a flora is more varied because a greater number of bacteria capable of deriving their energy from carbohydrate or protein can thrive in the intestinal environment than appears to be possible with the more or less obligately fermentative, lactic acid types.

9. The facultative flora, in which periods of carbohydrate ebb and flow is the dietary determinator, partakes of the acidogenic and aminogenic types respectively. At a given level of the tract, during these periods in which ample carbohydrate is present, the acidogenic activities of the flora are stimulated. During intervals of carbohydrate deficiency, the proteolytic activities are resumed.

10. A continuous, relative deficit of carbohydrate in proportion to the protein in the diet leads to the establishment of a proteolytic flora,

in which protein-liquefying organisms of the mesentericus and proteus types, together with smaller numbers of other similar organisms, are the prominent varieties met with.

11. The putrefactive products formed by the facultative and purely proteolytic types of intestinal bacteria comprise, in addition to unknown substances, aromatic amins, fatty acids, and aromatic nuclei of amino acids. Of these, histamin, tyramin and indol ethylamin are physiologically active even in minute amounts. Also, indol, phenol, paracresols, and skatol are formed in recognizable amounts. The subsequent fate of these substances within the body has already been discussed.

4. Exogenous Intestinal Infections

Bromatherapy.—Thus far, emphasis has been placed upon the principles underlying the general phenomena of bacterial metabolism, and applications of these principles to the elucidation of the mutual and reciprocal relations between diet and microbic response in the normal, or nearly normal, digestive tract.

An obvious extension of these principles to the therapeutics of exogenous and endogenous infections of the intestinal tract clearly presents itself. The need for specific therapy in intestinal infections is very great. The treatment of typhoid, cholera, dysentery, and other enteric diseases is expectant and supportive. There are no serums or antitoxins of proven value available, and chemotherapy is thus far unsuccessful. There is clearly an important place in clinical medicine for procedures of specific intervention which are in favor of the host, and antagonistic to the microbe, once infection is established. The prevention of infection does not of course enter into the discussion at this point.

A theoretical basis for specific intervention in intestinal bacterial infection resides in the relation of carbohydrate and protein sources of energy to the production of benign or noxious products of metabolism by pathogenic and parasitic bacteria. It will be remembered that diphtheria, dysentery, cholera, typhoid, paratyphoid, colon, proteus, and many other organisms form benign lactic acid from utilizable carbohydrate. They are potentially buttermilk bacilli so far as the chemical products of their growth are concerned, upon a suitable sugar diet. The removal of the carbohydrate, however, is immediately followed by the formation of nitrogenous, noxious products, many of which are poisonous.

Available evidence indicates that the same metabolic phenomena are involved in the intestinal culture *in vivo* and in the artificial culture *in vitro*. The underlying principles are identical. "Utilizable carbohydrate protects protein from bacterial decomposition."

This principle of the protective action of utilizable carbohydrate for

protein has been deliberately applied by the author in the treatment of bacillary dysentery. This is a severe infection of the intestinal mucosa incited by Bacillus dysenteriæ, of the Shiga, Flexner, or Flexner variant types. The effects are particularly severe in young children. The infective agent is restricted chiefly to the large intestine, and the organisms do not usually penetrate tissues deeper than the mesenteric lymph nodes. The essential specific feature of this treatment was to feed the patient lactose solution by mouth; glucose was injected subcutaneously for reasons to be detailed later.

Lactose was fed to permeate the entire digestive tract of the patient. By so doing the metabolism of the dysentery bacilli, and of the resident intestinal population as well, was shifted from protein to carbohydrate.[26] Two distinctly specific but related beneficial results were expected: To reduce the formation of toxins by the dysentery bacilli and to prevent the formation of indol and other putrefactive products by Bacillus coli and other intestinal organisms. The other beneficial effect hoped for would come from the acidification of the intestinal tract, due to the combined lactic acid generation of the entire intestinal flora, both pathogenic and parasitic. One of the significant results of lactose feeding was a reappearance of the normal nursling lactic acid bacilli; especially Bacillus bifidus and Bacillus acidophilus. In favorably progressing cases, these organisms rather rapidly became prominent. Their energetic lactic acid generating powers were of undoubted significance in rendering intestinal conditions intolerable for the acidophobic dysentery bacilli.[27, 28]

In addition to the oral feeding of lactose solutions, two other procedures for the administration of carbohydrate were practiced. One of these was an attempt to give glucose-lactose irrigations per rectum in the hope that some of the sugar would pass the sigmoid and enter the absorptive areas of the large intestine. This was soon abandoned. It proved to be annoying to the young patients without a proportionate gain. The other procedure was to infuse glucose solutions subcutaneously

[26] The generally accepted treatment for bacillary dysentery in young children at this time was starvation, upon the assumption apparently that the dysentery bacilli would gradually exhaust themselves. Water alone was given. It was obvious that all the intestinal microbes of necessity became proteolytic. The dysentery bacilli formed toxin, the colon bacilli indol, and the entire burden of detoxicating whatever of these nitrogenous products were absorbed from the intestinal tract fell upon the liver. The intestinal secretions and tissues furnished the requisite protein for the formation of these harmful products.

[27] The antagonistic effects of lactic acid production upon the viability of dysentery bacilli in the intestinal tract and dejecta have recently received unexpected substantiation in the Report of the Medical Research Committee.

[28] It is probable that lactic acid produced by microbic action within the alimentary canal and immediately in the presence of acidophobic bacteria is more effective in its action than an equal quantity would be brought from a distance. The neutralizing effect of salts and alkaline secretions would certainly change considerable amounts of the acid to the lactate, which is far less effective in its inhibition of microbic activity.

(Heilner, Allen).[29] It was found that young children could not retain even water by mouth when the dysenteric infection was severe. The dehydration of the tissues following the profuse diarrhea left the patients in a serious condition. The addition of glucose (Allen) to the saline infusion was devised to provide the tissues with an immediately utilizable source of energy as well as restore body fluid. It was also hoped that some of this glucose would be carried to the mesenteric lymph nodes or other tissues where bacteria might be growing within the body, and thus aid in a reformation of their metabolic products. This would mean, if it were realized, that the dysentery bacilli within the tissues would produce lactic acid in place of toxin so long as the glucose was available. In other words, these dysentery bacilli would become potentially lactic acid microbes.

An unexpected beneficial effect of lactose feeding was noticed. Children that constantly regurgitated water appeared to retain the lactose solution without difficulty. No explanation presented itself to account for this peculiar result.

At first sight, the selection of lactose as the carbohydrate for oral administration might be criticized on the ground that dysentery bacilli do not ferment this sugar. It should be emphasized, however, that lactose is more slowly absorbed from the digestive tract than any other sugar. This fact alone would increase manyfold the chances of permeating the entire intestinal canal with sugar.[30] Lactose is fermented by a majority of the normal intestinal bacteria and it will be remembered that one objective of the specific dietary treatment of toxic intestinal infection is to reduce intestinal bacterial proteolysis and augment lactic acid production. Acidogenesis should extend the entire length of the tract to be effective.

Lactose is apparently hydrolyzed in the intestinal mucosa by the enzyme lactase (Morse and Talbot). The products of hydrolysis are the hexoses, glucose and galactose, both of which are readily utilized for energy by dysentery bacilli. Inasmuch as the dysentery bacilli are growing in the intestinal mucosa, the advantages of liberating fermentable sugars there are obvious.

There is of course the possibility that the intestinal mucosa and immediately underlying tissues might be so injured by the poisons of the dysentery bacilli that the cleavage of lactose might be interfered with. It is not possible to disprove this contingency, but it may be stated that repeated examinations of urines from a series of cases treated in this manner were invariably negative with reference to the presence of re-

[29] These infusions were sterilized solutions of normal saline containing 2.5 per cent of Kahlbaum's chemically pure, anhydrous glucose. From two to four ounces were injected very slowly each day by the subcutaneous route for several days.

[30] Repeated, relatively small, feedings of lactose were prescribed rather than fewer, larger amounts. This was to insure the continuous presence of sugar throughout the intestinal tract.

ducing sugars. This would suggest that unaltered lactose failed to enter the tissues and blood stream in significant amounts.

It was soon realized that prolonged feeding of carbohydrate alone became harmful. This might confidently have been expected. Subsequent feeding with lactose-protein solutions were very well tolerated, no evil results attributable to the protein being observed so long as the carbohydrate was fed in amounts sufficient to insure a continuous flow to the lowest levels of the alimentary canal. Protein solutions without carbohydrate were found to be distinctly harmful.

The earlier cases of bacillary dysentery treated with the protein-lactose diet as indicated showed neither signs nor symptoms suggestive of harm arising from the liberal use of lactose. Somewhat later in the season, however, a striking instance of apparent harm attributable to lactose feeding presented itself. Inasmuch as this case presents details of importance in connection with the therapeutic application of dietary procedures to bacterial infections, the salient features will be briefly related.

A young child was convalescent from a severe attack of bacillary dysentery. It had passed successfully through the febrile and diarrheal stages of the disease upon the lactose-protein diet, and was apparently in such good condition that a more liberal regimen was indicated. Suddenly, without warning, the diarrhea reappeared together with the sanguineous, mucopurulent intestinal discharges previously observed. The clinical picture at first sight was one of a severe relapse. It was perfectly clear at this stage of the case that the lactose-protein feedings were distinctly harmful. They aggravated the patient's condition beyond reasonable doubt. It was observed that there was a slight difference in the constitutional symptomatology of this new attack. The patient was weakened very greatly, but the mental signs of profound toxemia were disproportionately slight as compared with those of the initial infection.

Repeated attempts to isolate dysentery bacilli from the feces and blood-stained mucus were unsuccessful at this time, although no trouble had been experienced in cultivating the organisms during the earlier diarrheal period. Gas bacilli [Bacillus aërogenes capsulatus or Bacillus welchii], however, were found in abundance. This had not been encountered in the dysenteric period of this case, nor had they been detected in other dysentery cases previously studied.

It is well known that gas bacilli are intolerant of preformed lactic acid, and with this in view well-soured buttermilk was administered in considerable amounts in place of the lactose-protein solution.[31] The symptoms, including the diarrhea, promptly abated, and the patient made an

[31] The use of well soured milk in cases of overgrowth of gas bacilli in the intestinal tract is an important example of the value of lactic acid milk in intestinal therapy. (Kendall and Smith, Hewes and Kendall.)

uneventful recovery. Subsequent examination of some of the lactose itself revealed an extensive contamination with the spores of the gas bacillus. Even so small an amount as ten milligrams sufficed to produce the well-known stormy fermentation of milk, and the development of the rancid odor characteristic of butyric acid. The injection of some of this milk into rabbits produced the characteristic distention, foamy liver and other signs of the Welch-Nuttall test, thus affording ample confirmation of the diagnosis.

The origin of the second attack of profuse diarrhea and the obvious relationship between the lactose and the aggravation of the symptoms in this case is very clear. The contaminated lactose was responsible for a direct implantation of spores of the gas bacilli in the digestive tract of this child.[32] These spores vegetated, and the gas bacilli multiplied rapidly. Inasmuch as Bacillus welchii is a most energetic fermenter of carbohydrates (Simonds, Blake), producing therefrom considerable amounts of butyric acid, it was in all probability the irritant effect of this acid upon the intestinal mucosa which caused the diarrhea. The absence of symptoms of toxemia is probably associated with the fact that butyric acid is not a toxin.

Two other patients, out of a number of dysentery cases undergoing the lactose-protein treatment, also developed gas bacillus diarrhea before the condition and its remedy were recognized. The administration of buttermilk was as effective in arresting the process in these cases as it was in the first instance. It should be mentioned in passing that gas bacillus diarrhea was so prevalent two years later among patients coming to the same hospital,[33] that it might be said to have existed in epidemic proportions (Kendall and Smith). It was not transmitted through lactose at this time, however, inasmuch as the infection existed prior to their admission to the clinic. Buttermilk proved to be as efficacious in the treatment of this group as it had been in the single cases just mentioned.[34]

To summarize: these dysentery cases and the gas bacillus infections arising from them are of interest from two viewpoints: First, because underlying principles of bacterial metabolism observed in culture and in the normal digestive tract have a direct bearing upon the specific dietary treatment of intestinal infections. Indeed, these principles are applicable to any infection where the anatomical relations to the host are such that full advantage may be taken of procedures which shall alter directly the metabolism of the microbe in favor of the host. These conditions

[32] All lactose solutions were subsequently sterilized in the autoclave, and all trouble from this source was at an end.

[33] Fifty-three out of a total of one hundred and thirty-five cases of severe diarrhea studied. (Kendall.)

[34] Similar cases have been seen in adults; also subacute and chronic types are occasionally met with. They are usually unrecognized, however. (Hewes and Kendall.)

usually may be predicted. Secondly, apparent exceptions to the practical working out of these principles may be caused by the abrupt development of latent, unrecognized organisms whose activities are favored by the regimen which controls those of the primary infective agent.

Such instances are not indicative of a failure of the principle; in fact, they are supplementary evidence of the correctness of the principle. They do suggest the necessity of a complete survey of the residual intestinal flora as a basis for the formulation of a correct dietotherapy. The gradual, or rapid, reëstablishment of a normal lactic acid flora, antagonistic to the development of the dysentery bacilli was readily determined by direct examination of the fecal flora, by cultural methods, and by chemical determinations of lactic acid. The shifting of the metabolism of intestinal organisms of the colon type was rendered probable. The shifting of the metabolism of the dysentery bacilli from toxicogenic to acidogenic was surmised. It could not be definitely proven.

The clinical results were, generally speaking, favorable. In no instance was any harm to the patient discernible. If it were possible to determine the initial damage to the patient by the dysenteric infection before specific food therapy was started, much more accurate statements could be made with reference to the probable beneficial effects of dietary treatment as a means of preventing subsequent poisoning. It may be stated without reservation that whatever was accomplished by direct dietary interference with the antagonistic activities of the dysentery bacilli was entirely in the interest of the host.

It is unfortunate that accurate chemical studies of the metabolism of at least a few of the cases so treated could not have been made. It was apparent that the dysenteric intoxication produced a deep-seated and unfavorable effect upon the metabolic processes of these patients.

The only available evidence is qualitative, not quantitative. The reduction of signs and symptoms of toxemia, the general suggestion of an amelioration of the severity of the infection, improvement of intestinal conditions with respect to digestion, and a tendency toward a relatively early recovery from loss of weight suggested that those same dietary factors which would theoretically restrict the pernicious activities of the invading microbe were favorable to the return of the host to a normal state.

Although metabolic studies upon dysentery cases fed with the lactose-protein diet are not available, the effects of the Shaffer-Coleman high calorie diet in typhoid fever offer a somewhat parallel condition. It has long been known that there is a "toxic destruction of body protein" in infectious febrile diseases, as typhoid, which is probably due in part to simple pyrexia, and in part attributable to the toxins originating with the organisms causing the morbid condition. The loss of tissue nitrogen and of body weight may be very considerable in typhoid fever, particu-

larly if the partial starvation diet principle be adhered to. Shaffer and Coleman sought to prevent this large loss of body nitrogen. They were led to prescribe a diet moderately rich in protein and fat, and extremely rich in carbohydrate, through a consideration of the well-established physiological dictum that carbohydrate spares body protein. They were able to keep several typhoid patients in approximate nitrogen equilibrium, but little below the normal, upon such a high calorie diet, and this form of dietary treatment has been rather generally adopted since the appearance of their studies.

The sparing action of the carbohydrate for body protein was manifested by the relatively slight losses in weight experienced by their patients. Another, and perhaps unexpected, result was observed. The toxic appearance, the "typhoid facies" of older days and accompanying symptoms of toxemia were noticeably reduced in those patients who were obviously benefited by the carbohydrate-rich diet. Among their conclusions, they state: "The 'toxic' destruction of body protein, as well as the destruction due to simple pyrexia in this disease [typhoid] may be either prevented or compensated for." "If, as seems probable from our results, the 'toxic' destruction of body protein may be prevented by a large carbohydrate intake, the mechanism of this 'toxic' destruction cannot be a direct [poisonous] injury to body cells and protein."

Bacteriologically, typhoid fever exhibits several similarities to bacillary dysentery. Both are initially intestinal infections. The dysentery bacillus rarely penetrates beyond the mesenteric lymph nodes, but typhoid bacilli usually invade the blood stream and may enter all the tissues. From the viewpoint of bacterial metabolism, a carbohydrate rich diet would be quite as much indicated to induce a reëstablishment of the intestinal flora, and a reformation of the metabolism of the typhoid bacillus in typhoid fever as is the case correspondingly in bacillary dysentery. The careful study of Torrey upon the intestinal flora of typhoid patients receiving the high calorie diet indicates that there is a clearly discernible change of the intestinal bacteria very similar to that observed in bacillary dysentery cases fed upon a lactose-protein diet. Torrey says, "On a diet consisting of a daily average of 50-100 grm. of protein, 75-100 grm. of fat, and 250-300 grm. of carbohydrate, including lactose, the intestinal flora tended to become converted into a fermentative type in which the dominant organism was Bacillus acidophilus. Patients exhibiting an initial fermentative flora of the aciduric type adapted themselves more readily to the high calorie diet of Coleman —in such patients the disease showed a marked tendency to run a mild course."

In addition to the changes noted in the types and metabolism of the bacteria of the intestinal tract, there is the additional possibility that a reformation of the metabolism of typhoid bacilli in the blood stream,

and possibly even in the tissues, may take place. Feeding a diet rich in carbohydrate certainly tends to keep the glycogen reservoir in the liver, muscles and elsewhere at a high level. The normal blood sugar, nearly 0.1 per cent in man, would likewise tend to be kept at or near its maximal level, through continuous repletion from the glycogen deposits and additions from the intestinal tract. One-tenth of one per cent of glucose continuously present in the general circulation would abundantly supply the minute requirements of the typhoid bacilli therein present. Under such conditions it is difficult to conceive of the failure of the organisms to utilize such a readily assimilable source of energy.[35] The living typhoid bacilli in the circulation would become potentially lactic acid bacilli. Furthermore, inasmuch as glucose appears to exist in simple solution in the plasma, it would diffuse readily into the tissues. It is possible, even probable, that the outside of necrotic foci containing the organisms in the spleen, liver and other organs would receive glucose. Whether this glucose would penetrate to the depths of such foci cannot be stated. A large carbohydrate intake stands in some very direct relation to the favorable progress of the disease. Sugar can not neutralize toxins, however, although they do prevent the formation of toxins in many well known instances.

The diminution in signs of toxemia and the "prevention of or compensation for toxic destruction of protein and body cells," noticed by Shaffer and Coleman, has significance in the light of the effect of utilizable carbohydrate upon the metabolism of the typhoid bacillus. It must be recognized that the "toxic" action observed in typhoid fever rests ultimately with the growth of the organisms, because they alone incite the disease, typhoid fever. An amelioration of the signs and symptoms of toxemia suggests direct interference with the formation of the toxic agent, whatever it may be. Looking at this reduction of toxic phenomena from the viewpoint of the shifting of the metabolism of the typhoid bacillus from proteolytic [toxicogenic] to fermentative, it will be seen that the continuous supply of glucose, furnished by the Shaffer-Coleman high calorie diet, provides exactly the chemical basis for its accomplishment.

Attention is redirected again at this point to the general theory, attested to by physiologists, that "utilizable carbohydrate spares body protein" and the essential agreement of the physiological and bacteriological response under parallel conditions.

[35] Metabolic studies of typhoid bacilli in sterile, defibrinated blood, and in sterile blood serum (containing the normal percentage of blood sugar) have shown that the protein constituents are left practically intact until the glucose is fermented. In this connection, the observations of McGuigan and von Hess that glucose may be obtained from the circulating blood in animals by dialysis through collodion membranes is of significance. They conclude: "Dialysis of normal circulating blood shows the blood sugar to be entirely free and to exist in simple solution in the water of the plasma." Sugar in this state is available for energy in the blood stream by typhoid, or in fact any other, bacteria which can utilize it.

Summary and Conclusions

Other infectious diseases of the digestive tract of the toxicogenic type, as paratyphoid fever, Asiatic cholera, coli colitis, and invasion by the meat poisoning bacteria, are equally available for carbohydrate therapy. The general principle involved is the same. The objectives to be attained are:

1. The establishment of a lactic acid [fermentative] intestinal flora in which Bacillus acidophilus or Bacillus bifidus, or both, become dominant.
2. The shifting of the metabolism of the normal, facultatively proteolytic organisms to the fermentative side.
3. The shifting of the metabolism of the invading organism from the toxicogenic [proteolytic] to the fermentative side.
4. To be certain that organisms productive of abnormal fermentative products, as gas bacilli, are not resident in the intestinal tract in numbers sufficient to become offensive when the carbohydrate regimen is established.
5. To administer carbohydrate in amounts and at intervals sufficient to keep the entire digestive tract, and particularly the lower levels, continuously permeated with the requisite amount and kind of sugar.

Properly carried out, this bromatherapeutic method of specifically influencing infection will result in several important contributions to the welfare of the patient.

The reëstablishment of a normal acidogenic flora will create intestinal conditions unfavorable to the development of those invaders which are in the alimentary canal.

The fermentative shifting of the metabolism of the members of the facultative group will prevent the formation of indol and other bacterial decomposition products of the amino acids. This will lessen materially the work of the liver.

The fermentative shifting of the metabolism of the invading organism will make it potentially a lactic acid bacillus in place of a toxicogenic organism. The abundant supply of carbohydrate will tend to reduce the loss of body protein to a minimum, thus conserving the strength of the patient. It will be seen that this procedure of bromatherapy is equally indicated from the physiological, bacteriological, and biochemical viewpoints. It is specifically in the interest of the host and equally directly in opposition to the baneful activities of the parasite. It must be realized that bromatherapy, as outlined above, is subject to the same general limitations as any other form of therapy. Damage already accomplished

before dietary procedures are begun can not be rectified, nor can the influence of this damage upon the subsequent progress of the disease be determined with precision.

Perforations, hemorrhage, or other complications, can not be influenced to any extent, nor can they be prevented, in all probability, by such measures. Some time the specific poison or poisons of the cholera-typhoid-dysentery group, as well as those of other intestinal invaders, may be discovered, and more specific antidotes discovered for them than are now available. In the meantime, the possibility of reforming, but not of annihilating, these microbes appears to be the most direct method of restricting their activities. The dietary route, both in the interest of the metabolism of the patient and the reformation of the metabolism of the microbe, is the procedure which thus far has had experimental justification and practical application.

SECTION VII

Actions of Drugs and Therapeutic Measures

The Effects of Certain Drugs and Poisons upon the Metabolism . . . . . *Henry C. Barbour*

Water and Salts—Deficiency of Water—"Mineral Waters"—Salts—Saline Cathartics—Other Cathartic Drugs—Sodium Chlorid—Potassium, Lithium and Other Salts—Bromids—Iodin and Iodids—Salts of Organic Acids—The Alkaline Earths—Calcium Deprivation—Calcium in Leprosy—Calcium in Tetany—Other Effects of Calcium, etc.—Aluminium—Acids and Alkalies—Neutrality Regulation—Acids—C.C. of CO Bound by 100 C.C. of Plasma—Total Metabolism—Purin Metabolism—Boracic Acid and Borax—Oxygen and Asphyxiants—Oxygen Deficiency—Carbon Dioxid—Carbon Monoxid—Other Blood Poisons—Cyanids—C.C. CO in 100 C.C. Blood—Phosphorus, Arsenic, Heavy Metals, etc.—Organic Phosphorus—Cod Liver Oil—Arsenic and Antimony—Mercury—Chromates—Lead, Platinum, Copper, Zinc—Radium—Narcotics—General Anesthetics: Chloroform and Ether—Hypnotics—Alcohol—Opiates—Antipyretics—Quinin and Its Congeners—Ethylhydrocuprein—Cinchophen (Atophan)—Ammonia, Amins, Alkaloids, Purins, etc.—Ammonia—Hydrazin—Ethylenediamin—Iso-amylamin, Phenylethylamin, and Tryamin—Beta-tetrahydronaphthylamin—The Amino Acids—Atropin Pilocarpin, etc.—Strychnin—Some Other Convulsants—Camphor—Santonin—Curare—Cocain—Purins—Endocrine Drugs—Epinephrin—Thyroid Gland Substance—Pituitary Substance—Anterior Pituitary Lobe—Other Gland Products—Thymus Gland—Parathyroid Gland—Spleen—Prostate Gland—Testis—Pineal Gland.

The Effects of Certain Drugs and Poisons upon the Metabolism

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I. Water and Salts

Water taken in excess of demand is promptly eliminated from the body, but its removal may alter the mineral balance or disturb the relative proportions of the ions. The metabolic changes may include a temporary increase in the urinary nitrogen, due apparently not only to "flushing," but also to some extra protein breakdown (Hawk).

The effects of water in moderate amounts upon the total metabolism were first investigated by Bidder and Schmidt (1852), who reported them negligible, and F. G. Benedict employing highly perfected technique has recently shown that normal adults may ingest 500 c.c. of water at room temperature without altering the basal metabolism. Larger amounts may prove stimulating, but 200 c.c. of water given per os did not alter the metabolism of Lusk's 9.3 kilo dog.

Such water ingestion in health does not affect the body temperature.

Large amounts of water taken with proteins and fats do not influence the absorption of the latter from the alimentary canal (Edsall).

Deficiency of Water.—Water deprivation as well as excess results in an increased protein destruction; the excess metabolites do not, however, appear in the urine until its checked flow has been restored by renewed intake of fluid. (Straub.)

An adequate water content of the blood is so essential to the various processes of heat elimination that any considerable dehydration of the body (because of the diminished blood volume) results in fever. Salt fever (see below) has been thus explained by Balcar, Sansum, and Woodyatt, who themselves produced extraordinary temperature elevations in dogs by dextrose dehydration (in one case 125° F. was observed!). Conversely water often serves as an antipyretic agent. The fever of the newborn, formerly accepted as physiological, can be prevented entirely by an occasional spoonful of water.

The effects of water deficiency are further discussed in connection with salt action.

"**Mineral Waters.**"—Natural spring waters have been so long and extensively exploited that the tendency to ascribe to them some occult therapeutic value still lingers. No evidence exists, however, that their employment (most successful at their source) is associated with effects beyond those attributable to the individual mineral ingredients (see below) or to psychic, climatic and hygienic factors.

Salts.—The effects of salts upon the metabolism fall into two categories, namely, those due to (1) "salt action" (chiefly osmotic processes) and (2) the action of individual ions. Pertaining chiefly to the first group are the effects of the

Saline Cathartics.—Poorly absorbable salts, of which the sulphates of sodium and of magnesium are noted examples, act as dehydrating agents, their systemic effects being therefore essentially those of water deficiency. This applies as well to parenteral administration, where diuretic instead of cathartic action results.

Body Temperature.—In connection with the therapeutic employment of saline cathartics significant temperature changes are not seen. Hay was unable to substantiate the reputed "cooling effect" in fevers. On the contrary, where the dehydrating effect becomes pronounced, some increase in temperature may be anticipated (salt fever).

Total Metabolism.—It was claimed by Loewy(*b*) that saline cathartics augment the total metabolism, this effect being attributed to increased peristalsis. Others, on the basis of Hay's theory considered that the alleged increase in the total metabolism was due to the work involved in the active "secretion" of water into the intestine. However, after Wallace and Cushny showed that osmotic factors alone will account adequately for the passage of fluid into the bowel, it was not surprising that Brodie, Cullis and Halliburton should find that hypertonic magnesium sulphate causes no increase in the oxygen consumption of the intestine itself. Ultimately F. G. Benedict demonstrated that oral therapeutic doses of the saline cathartics do not measurably increase the total metabolism of healthy individuals.

An instance of increased oxygen consumption in a single organ is, however, seen in the results of Bainbridge and Evans, who, in a contribution to the secretory theory of diuretic action, describe an increase in the gas consumption of kidneys subjected to the action of sodium sulphate.

Protein Metabolism.—The protein catabolism may be increased by saline cathartics when exhibited in amounts sufficient to deplenish the body's stock of water.

Fat Metabolism.—The habitual use of salines is frequently efficient in reducing the weight in obesity. Many of the natural mineral waters have acquired a reputation in such cases. Their action appears to be due in part to their hindering the absorption of proteins and fats (Hay), in part to a depletion of the body fluids by the salt action. Saline cathartics are

said to increase the percentage of butter fat in cow's milk, but this is not a dependable result (McCandlish).

Carbohydrate Metabolism.—Franck attributed "salt glycosuria" (discussed below) to polyuria, but other explanations are better supported by the evidence.

Mineral Metabolism.—Chiari has suggested that since all cathartic ions are antagonistic to calcium the action of the saline cathartics may be explained by assuming that the calcium normally present keeps the intestinal cells in a state of low permeability.

The specific systemic effects of neither the magnesium ion of Epsom salts nor the tartrate ion of Rochelle salts are seen after oral administration. For a discussion of these see under "Alkaline Earths" and "Salts of Organic Acids," respectively.

Other Cathartic Drugs.—The effects upon the metabolism of those cathartic drugs which act primarily by stimulation of peristalsis have never been adequately investigated.

Aloin.—This drug was administered to mammals and birds by Berrar, who observed a marked increase in the energy exchange accompanied by a rise in temperature. The nitrogen excretion (especially urea in mammals and uric acid in birds) was also augmented.

Sodium Chlorid.—Because of the high normal sodium chlorid content of the body (150-300 grams according to Magnus-Levy) and the fairly delicate chlorid-regulating mechanism, a considerable salt intake is required before effects upon the metabolism are noted. In general the effects of sodium chlorid upon the metabolism are probably due rather to osmosis than to specific ion actions.

Mineral Metabolism.—The skin acts as the chief of several chlorid depots, storing or releasing salt according to need.

Rosemann(*e*) found the entire chlorid content increased by 100 per cent when dogs were given highly salted food. The chlorid threshold of the plasma is said to be 5.62 grams per liter. According to MacLean if the concentration falls below this level no chlorid is excreted; if it exceeds it the excretion varies as the square of the excess.

Holt, Courtney and Fales(*c*) have investigated in children the effects upon the mineral metabolism of 200 c.c. injections, by hypodermoclysis, of physiological saline. Salt and water are retained for several days. The effects are most marked in conditions where salt and water deficiency exist, as in acute diarrhea, marasmus and protracted vomiting. The retention is accompanied by much symptomatic improvement. The changes in magnesium, calcium, phosphorus, and potassium metabolism were also followed by Holt and his collaborators, but no uniformity could be detected. A "balanced" salt solution (potassium and calcium chlorids being added) gave results not differing from those of the sodium chlorid solution alone.

Water Metabolism.—The urine is increased in amount by sodium chlorid, as by other solids which the kidney eliminates. All salts readily absorbable from the alimentary tract act therefore as diuretics. It is well known that salts, especially sodium chlorid, play an important rôle in the movement of fluids everywhere in the body, as in secretions, effusions and edemas.

Body Temperature.—The phenomenon known as salt fever came to light through observations of pediatricians, notably Finkelstein and Schaps, who observed a rise in the body temperature of infants subsequent to oral or subcutaneous administration of saline solutions. In adults Bingel obtained less constant results from one liter injections of 0.9 per cent sodium chlorid; the maximum temperature changes varied all the way from $-0.3°$ to $+2.5°$ C., the fevers greatly predominating, however. When a solution containing NaCl 1.8, $CaCl_2$ 0.24, KCl 0.42 and $NaHCO_3$ 0.2 gm. in one liter was given the temperature increases were also frequent and pronounced.

To account for salt fever a specific sodium ion effect has been claimed by many; Burnett and Martin, for example, were able to prevent its appearance by antagonizing the sodium with proper amounts of calcium. While the above-mentioned results of Bingel in no wise disprove the sodium ion theory, some observers, as Rolly and Christjansen, find hypertonic (3 per cent) saline more effective than isotonic, indicating that salt action is at least an important factor.

Heubner(*b*) studied the effects of intravenous saline injections in rabbits and states that while 0.1-0.3 milligram were pyretic, doses of twenty times this magnitude gave a prompt temperature decrease. This latter effect was possibly associated with protracted dilution of the blood. Having obtained negative effects with his Ringer solution injections Heubner favors the sodium ion theory.

Extensive work upon salt fever has been reported by Freund, who pointed out a parallelism between sodium chlorid and epinephrin effects; under similar conditions he produced both fever and glycosuria by injecting either of the two agents intravenously. From these and like results he concluded that "the disposition to sodium chlorid fever" is equivalent to a state of hyperirritability of the sympathetic nervous system.[1]

Freund also obtained sodium chlorid fever by oral administration in rabbits, 1.5-2 grams giving the best results; 3 grams frequently, and 4 grams always, reduced the temperature (as was the case with Heubner's larger injections). He pointed out that the oral experiments dispose effectively of a rather widespread contention that salt fever might be attributed entirely to the "water infection" which intravenous injections

[1] Epinephrin, salt and sugar fevers lend themselves to a single interpretation: loss of water from the blood.

of stale distilled water sometimes produce. He also failed to obtain salt fever with intravenous Ringer solution.

In the hands of the present author 20 c.c. per kilo of dextrose-free Locke solution made with water freshly redistilled from glass gave the same results as physiological sodium chlorid solution,—a temperature rise of over 1° C. when either was injected into the ear veins of normal rabbits. (In both cases a fall of 0.2° C. during the first twenty minutes was obtained.) Furthermore Barbour and Howard with 8 c.c. per kilo of a similar Locke's solution intravenously injected were able after an interval of fifteen minutes to superimpose a steep salt fever rise upon the plateau of the "coli fever" curve in dogs.

It certainly appears probable that salt fever is due chiefly to a loss of water from the blood, whether the water be drawn chiefly to the kidneys, to the site of salt administration or, on account of disturbed capillary permeability (for which complex ion interchanges might be responsible), to other tissues.

Hashimoto has shown that salt fever is less readily produced during artificial warming of the "heat centers" in rabbits. The contention that salt fever results from irritation of the "heat centers" by products of the interaction of sodium with the tissues has not, however, been substantiated.

The salt fever riddle has important bearings upon infectious fevers, in many of which disturbances of the water and chlorid metabolism are well recognized.

Total Metabolism.—Sodium chlorid increases oxidations slightly whether given per os or subcutaneously. Freund and Grafe found that the heat production was augmented 8 per cent as against 22 and 28 per cent increases after Ringer and dextrose solutions, respectively.

Raeder found in the case of subcutaneously injected saline solutions that hypertonicity favors the increase in oxidations. This may be merely the result of a higher body temperature or it may be due in part directly to osmotic action.

Tangl found the oxidations increased by sodium chlorid given per os to curarized animals without kidneys. This would tend to relegate both central nervous and diuretic factors to a position of secondary importance in salt fever questions. Apparently dehydration into the stomach would account for Tangl's results.

Nitrogen Metabolism.—In salt fever Freund and Grafe found 20 to 45 per cent increases in the excretion of urinary nitrogen(*b*). (Compare the effects of water drinking described by Hawk.) Straub(*b*), however, states that sodium chlorid in non-dehydrating concentrations exerts a slight sparing effect upon the nitrogen metabolism; similar results have been obtained with the nitrate, acetate, carbonate, sulphate or phosphate of sodium (Loewi).

Salt Glycosuria.—This phenomenon, which has been investigated chiefly in rabbits, bears an undoubted relation to salt fever. It was discovered in 1871 by Bock and Hoffmann as the result of injecting into the arterial circulation of rabbits large amounts of 1 per cent sodium chlorid. Others have added to the list of glycosuria-producing salts the acetate, bicarbonate, phosphate, succinate, valerianate and sulphate of sodium. Kleiner and Meltzer(*b*) have shown that the last mentioned produces no hyperglycemia, thus differing from magnesium sulphate (see below).

A number of authors have considered the possibility that salt acting through the central nervous system may exert a stimulating influence upon the adrenal glands. This would accord with Freund's parallelism between the glycosurias and fevers caused respectively by salt, sugar and epinephrin. Furthermore, Waterman and Smit found an increased epinephrin content in the blood in salt glycosuria, while Stewart and Rogoff(*a*) have recently shown that concentrated sodium carbonate solutions increase the epinephrin output from the adrenals. Mobilization of glycogen by salt through the agency of these glands would thus seem to be strongly suggested.

However, MacGuigan's demonstration that epinephrectomy in cats is without influence upon salt glycosuria (although in dogs the operation does make the glycosuria more difficult of accomplishment) seems to exclude the adrenals as the prime causative factor.

Fischer(*a*) found that the intravenous injection of sodium chlorid (one-sixth molecular or stronger) causes glycosuria in rabbits after a certain latent period. Weaker solutions exert less effect or none at all. The addition of calcium chlorid prevents or puts an end to the appearance of sugar; the latter reappears, however, after returning to pure sodium chlorid. Fischer was inclined to exclude osmosis as a factor because urea, glycerin and alcohol all failed to produce glycosuria. Since salt injections into arteries leading directly to the brain caused quicker and more profound results the theory of a central action was favored.

The blood sugar in salt glycosuria was investigated by Underhill and Closson, who found it diminished. Underhill and Kleiner(*b*) were able to inhibit the hypoglycemia and glycosuria as well as the accompanying polyuria by calcium chlorid whence they concluded that the latter restores the retaining power of the kidney for glucose which sodium chlorid apparently impairs. The calcium injection even made the kidneys unusually impermeable to injected glucose which affords a counterpart to Pavy and Godden's experiment in which sodium chlorid was shown to reduce the tolerance of rabbits towards injected sugar. Salt glycosuria was therefore attributed by Underhill and his co-workers to increased renal permeability; dyspnea was invoked as an additional factor, for in the case of *arterial* injections hyperglycemia and glycosuria without polyuria were

noted. Recently McDanell and Underhill have accomplished further work, showing that $\frac{M}{2} - \frac{M}{6}$ sodium chlorid produces glycosuria with neither relative nor absolute hyperglycemia.

Hyperglycemia has also been found by others, but only when concentrated saline solutions were injected. According to Wilenko intravenous injection of 20 per cent saline produces by stimulation of the central nervous system a hyperglycemia in which the muscles and probably the liver lose glycogen.. He concluded that the nervous stimulation is a sodium ion effect and that owing to osmotic factors the permeability of the kidney is first increased and then decreased. Hirsch also obtained hyperglycemia from concentrated (10 per cent) sodium chlorid; 2.5 per cent or more dilute solutions did not increase the blood sugar nor did sodium carbonate, sodium acetate or calcium chlorid. He favored the central nervous system theory, which, however, fails to account for the non-appearance of hyperglycemia with the dilute injections.

Burnett has demonstrated the inhibiting effect of potassium salts upon the glycosuria produced by sodium salts, thus adding weight to the importance of the ions wherever the action may be exerted.

That the point of action of the ion antagonism in salt glycosuria is renal seems difficult to doubt in the light of the recent experiments of Hamburger, Brinkmann and their co-workers(*a*) (*b*). These investigators have studied the permeability of the glomerular membrane in the frog (the tubules being anatomically separated therefrom in this animal). They have demonstrated clearly the power of the glomeruli to retain free dextrose, but have also shown that this power depends upon the maintenance of a very delicate ion balance in the perfusion fluid. While Hamburger's attention was confined more to the calcium-potassium relations and the bicarbonate requirement, it is obvious that conditions which alter the sodium-ion concentration are likely to disturb seriously the entire ion balance. This applies to ion physiology in general, as shown by Loeb, and to the instance of salt glycosuria in particular, as shown by the calcium antagonism of Fischer and of Underhill and the potassium antagonism of Burnett.

An interesting practical deduction which Hamburger makes is that the oatmeal treatment in diabetes mellitus may owe its value to bolstering up the retaining power of a glucose-surfeited glomerular membrane by the excess of potassium ions contained in that food. Hamburger's(*b*) work should lead to a new understanding of the various types of renal glycosuria, of which sodium chlorid glycosuria appears to be a notable example.

Salt Starvation.—A deficient salt intake leads to emaciation, the occurrence of acetone in urine and breath and other untoward symptoms. A generally lowered mineral excretion results. The nitrogen balance appears to be but little affected (Rosemann(*e*)).

Potassium, Lithium and Other Salts.—Outside of the importance of the potassium ion in preserving the retaining power of the glomeruli for dextrose practically no metabolic effects peculiar to potassium salts have been demonstrated. They are, however, said to antagonize the beneficial effects of calcium in parathyroid tetany (MacCallum and Voegtlin). These ion relations in tetany appear, however, to concern rather the irritability of muscle than the metabolism (Zybell, cited by Gamble).

Salts of lithium, rubidium, cesium, etc., are more toxic than the corresponding sodium or potassium salts. Specific metabolic effects have not been shown. Lithium does not form soluble urates in the presence of sodium or potassium, which fact disposes of its formerly alleged value in gout.

Bromids.—Chlorids and bromids mutually increase the elimination of one another. The theory of Wyss, however, that the therapeutic action of bromids is due to chlorid-deprivation is not sound, for simple dechloration exerts no antispasmodic effect. Furthermore, Janusche has shown that bromid depression can be neither efficiently antagonized by sodium chlorid administration nor reënforced by chlorid-poor food.

Bromids appear to reduce the edema of uranium poisoning, stimulating the retarded water and chlorid excretion (Laeva).

Boenniger claimed that bromid administration may save animals from chlorid starvation and replace completely the chlorid of the serum, but Bernoulli finds that the replacement by bromid of more than 40 per cent of the blood chlorid is generally fatal.

The protein metabolism remains uninfluenced even by large doses of bromids; for example, Chittenden and Culbert found it unchanged during ten days in which 46 grams of potassium bromid were given. In experiments upon himself Schultze observed an average reduction of 19 per cent in the phosphate excretion following 10 gram doses of potassium bromid; the excretion of nitrogen and sulphur, however, remained unaffected. Japelli(*a*) in more recent investigations found little or no effect upon the total nitrogen or phosphorus excretion, but observed a diminution in the uric acid accompanied by an increase in the purin bases.

Schabelitz has studied chronic bromism, which leads to emaciation. The administration of chlorid, in addition to stopping the drug, was found to hasten the disappearance of the symptoms.

Iodin and Iodids.—In very exact experiments Magnus-Levy was unable to detect any influence of potassium iodid or of iodin upon the total metabolism of either healthy or obese persons; 3-10 grams of potassium iodid or 4-10 drops of tincture of iodin were given daily over a period of weeks. Magnus-Levy further found iodin inactive in a case of myxedema in which the metabolism had been notably stimulated by iodothyrin. The only case in which he observed any increase in the total oxidations under iodids was that of an emphysematous patient in whom

the drug aroused a febrile reaction towards the close of each day. Magnus-Levy's negative results have been confirmed.

According to Christoni iodids may increase the excretion of urea, total nitrogen, uric acid, purin bases and chlorids.

Hunt and Seidell have shown that thyroid preparations are efficient in treatment in proportion to their iodin content.

Recent investigations upon the catabolic effect of various thyroid preparations appear to indicate that the increase in nitrogen elimination is proportional to their iodin content (Courvoisier, Peillon, Lanz).

Swingle maintains that iodin is the specific agent by which amphibian metamorphosis is accelerated when thyroid substance is fed.

Treatment and Prevention of Goiter.—Iodin becomes rapidly fixed in the thyroid; Marine and Rogoff(*b*) ascertained that the fixation end-point is reached five minutes after the intravenous injection into dogs of 50 milligrams of potassium iodid.

The careful administration of iodids causes a regression of active thyroid hyperplasia into the relatively harmless colloid type of goiter. For this purpose Marine(*a*) advocates syrup of ferrous iodid in doses gradually increasing from 0.3 to 1.2 c.c. per day.

The prevention of goiter by iodid has been definitely achieved by Kimball and Marine. They fed 2-4 grams sodium iodid (in ten equal doses) to school girls in Akron, Ohio, none of whom became goiterous. Twenty-six per cent of the control series of girls (according to expectation in that locality) showed definitely enlarged thyroid glands. Hunziker suggests the use of iodin-rich manures in regions where goiter is endemic and vegetation lacks the standard proportion of iodin. He further suggests the admixture of iodin with table salt.

Toxic effects are often seen in goiterous (especially Basedow) patients if the large doses of iodids commonly employed in other diseases are administered. The symptoms, which include emaciation and fever, are detailed by Oswald(*b*). Acute untoward effects of intravenous or subcutaneous injections of iodids include pulmonary exudation and edema besides pericardial effusion. According to Chiari and Janusche these may be prevented by calcium injections.

The destructive effect of iodids upon pathological growths, particularly gummata, has never been completely explained. Jobling and Peterson believe that they restrain the antitryptic activity of serum and tissues, thus permitting autolytic digestion to proceed. Full doses of iodid in man greatly lower the anti-ferment index of the serum.

Salts of Organic Acids—*Oxalates.*—Salts of oxalic acid possess no known therapeutic value. Many of their effects are doubtless due to calcium deprivation. Sarvonat and Roubier found that sodium oxalate diminishes the calcium content of the soft tissues before affecting the bones.

Corley maintains that the total metabolism is much depressed in oxalate poisoning and that there is a lowering of the respiratory quotient. Wichern has described anuria followed by polyuria. Asphyxia, pyrexia and glycosuria may also occur.

Tartrates.—Intravenous injection of tartrates (Rochelle salts), in rabbits inhibits markedly the excretion of urea, but chlorid excretion remains unaltered. Underhill, Wells and Goldschmidt showed that this is due to a specific effect upon the renal tubules.

To be similarly accounted for is the fact that tartrates diminish the intensity of various glycosurias, e. g., phlorhizin (Baer and Blum), epinephrin and dextrose glycosurias (Starkenstein).

Benzoates.—These are of importance in view of their use for the preservation of food. Chittenden, Long and Herter in an exhaustive study could demonstrate no effects upon healthy individuals if the ingestion of one-half gram per day was continued for weeks. Even four-gram doses were rarely injurious. The body weight did not diminish, the digestion and utilization of fat and protein as well as the nitrogen-balance and partition and the quantitative composition of the urine all remained normal.

In man benzoic acid ingested in doses up to ten grams per day is excreted almost quantitatively as hippuric acid (Dakin).

Large doses of benzoates (eight grams per day in man) increase the urinary urates at the expense of the blood (Denis(*d*)). During the period of maximum hippurate excretion, however, Lewis and Carr observed a marked decrease in uric acid excretion. This was seen after seven to eight grams of benzoate, but could not be produced by the direct administration of hippuric acid.

Creatinin metabolism is not affected by benzoates.

Acetates and Citrates.—Acetates and citrates are converted into bicarbonates in the tissues, then acting as alkaline diuretics. (See Chapter III.)

II. The Alkaline Earths

Calcium, Magnesium, etc.

Mineral Metabolism.—That calcium administration in man may increase the calcium store of the tissues and blood was shown by Voorhoeve(*c*). Heubner and Rona state that intravenous injections of calcium salts in cats will double or triple the calcium content of the blood; this, however, returns to normal within two hours.

Givens(*a*) (*b*) has shown that calcium lactate in man increases the calcium excretion in the urine, but not to the same extent as milk does. On the other hand, magnesium citrate does not increase the magnesium excretion.

The calcium content of the serum in tuberculosis was investigated by Halverson, who found that it is not increased by a milk diet.

Magnesium, as shown by Malcolm, lessens lime deposition in young animals. In accord with this fact Mendel and Benedict found that it increases the urinary calcium. The presence of phosphates, however, inhibits the increase by magnesium of calcium excretion in the urine (Steenbock and Hart).

Strontium administration to young animals disturbs bone formation. Lehnerdt showed that the osteogenetic tissue is stimulated, but the bones become imperfectly calcified, the calcium being deficient and the strontium incompletely deposited.

In the magnesium narcosis of Meltzer and Auer (which can be antagonized by calcium chlorid injections) Stronsky has studied the plasma and has shown an increase in the magnesium content while the calcium content is diminished.

C. Mayer maintains that the chlorids of the alkaline earths tend to increase urinary acidity. This is contradictory to the usual holding since part of the phosphate is deflected by calcium, for example, to the intestines.

Calcium Deprivation.—In young animals fed on a calcium-poor diet the bones may contain a normal percentage of calcium, but what little new bone is formed is thin, pliable, deformed and fragile (E. Voit). It contains more water, sodium and potassium, while the magnesium is not materially increased. The percentages vary in different parts of the skeleton. Weiser describes the animals as undersized, with poor appetite and defective nutrition. Luithlen has increased or decreased the calcium content of the bones in rabbits by feeding, respectively, a green or an oat diet. (See also Oxalates.)

In studies of multiple exostosis Underhill, Honeij, and Bogert found evidence suggesting that a restriction of the calcium and magnesium intake during the stage of proliferative cartilage changes would be beneficial.

Calcium in Diseases of Bone Deficiency.—Rickets, being due not to deficient calcium income, but to derangement of the processes of assimilation, the therapeutic inefficiency of calcium in this disease has been generally upheld (Klotz(*b*)). This does not mean, however, that none of the administered calcium is retained. Schloss(*b*), for example, reports in a series of eighty experiments upon rachitic children the following results:

	Retention of CaO gram per day.
Fore period	0.032
With calcium administration	0.297
With cod liver oil	0.167
With cod liver oil and calcium	0.354

In respect to enhancement of the cod liver oil effect calcium appeared superior to phosphorus, which, when given with the oil, did not exhibit any influence upon the calcium retention.

Triacalcium phosphate Schloss found slightly better than calcium acetate and equal in retention value to some other organic calcium preparations.

In the florid stages of rickets a high magnesium retention was noted. This fell rapidly as the calcium retention increased, presumably owing to medication.

Gamble cites the following figures relative to calcium retention in osteogenesis imperfecta:

Author	*Age of patient*	*Medication*	*Retention of CaO gram per day*
Bamburg & Huldschinsky	3 months	none	0.042
		cod liver oil + phosphorus	0.089
Bookman	3 months	none	0.054
		calcium lactate with food	0.402
Orgler	3 months	none	0.130-0.210
Schabad	7-10 years	none	0.176
		cod liver oil + phosphorus	0.340
		cod liver oil + phosphorus + calcium lactate	0.338
		cod liver oil + phosphorus + calcium lactate	(neg. balance)
		thyroid substance	(low or negative)
		Fowler's solution	0.403
Herbst		Fowler's solution	0.382
			0.418

Schabad(*c*) prefers arsenic to other medication in this condition, but his results and those of others suggest that wide variations in calcium retention occur independently of medication.

The conditions which govern calcium retention and assimilation in pathological states are practically unknown.

Calcium in Leprosy.—Recent investigations of Underhill, Honeij and Bogert suggest that in leprosy administration of calcium may be of benefit in retarding or arresting the progress of the characteristic bone changes.

Calcium in Tetany.—Parathyroidectomy is followed by clonic convulsions with fever. MacCallum during such an attack in a dog observed the temperature increase from 39° to 43.2° C. The administration of calcium acetate stopped the convulsions in a few minutes and within one-half hour the temperature fell to 38.9°. MacCallum and Voegtlin also

reported success with calcium injections in a number of cases of human tetany.

The precise relationship of the calcium metabolism to parathyroid tetany has, however, not yet been demonstrated. Wilson, Stearns, Thurlow and Janney as well as McCann and others have shown that removal of the parathyroid is followed by a condition of *alkalosis*. This is neutralized by the acid production incident to tetany, or the tetany may be prevented by $\frac{M}{7}$ HCl intravenously injected. Now calcium salts have been found to lower the oxygen-combining power of the hemoglobin as well as the alveolar carbon dioxid tension, both of which effects may also be induced by acids. Calcium is, therefore, in some respects adapted to reduce a condition of alkalosis.

Howland and Marriott(*b*) have contributed to the question of the calcium metabolism in infantile tetany by demonstrating that in this condition the calcium content of the blood is approximately halved. Their average figure for eighteen cases was 5.6 milligrams in 100 c.c., the lowest being 3.5 milligrams. The corresponding normal figure was found to be 10-11 milligrams. They do not wholly accept the alkalosis theory. Calcium chlorid per os was found effective in increasing the serum calcium coincidently with cessation of the symptoms, although in most cases the normal calcium content was not attained.

Brown, MacLachlan and Simpson have recently found that intravenous injections of 1.25 grams calcium lactate may keep the signs of tetany in abeyance for from seven to ten hours. They state, however, that no permanent effects are obtained unless the treatment includes cod liver oil and phosphorus. The value of these last as regards rapid reduction of the symptoms is enhanced by the addition of the calcium. Cod liver oil and phosphorus produce within about two weeks an increase in the calcium content of the blood.

Uhlenhuth(*a*) has succeeded in suppressing with the lactate of calcium or magnesium as well as with a weak milk solution the tetany exhibited by thymus-fed salamander larvæ. The development of permanent paralyses and contractures is not, however, prevented. This form of tetany (which Uhlenhuth believes to be a true parathyreoprival tetany) is therefore shown to be due to a specific toxic substance not perfectly antagonized by calcium, magnesium or milk.

Marine(*b*) has shown that parathyroid hyperplasia of the fowl (which is produced by feeding maize or wheat) can be retarded by feeding calcium.

When the prevention or treatment of the dysparathyroidisms shall have been perfected, one feels justified in believing that a prominent rôle therein will be played by calcium.

OTHER EFFECTS OF CALCIUM, ETC.

Water Metabolism.—The effects of the calcium ion upon water exchanges in the organism are very imperfectly understood.

Many of them may be ascribed to diminished permeability of the kidneys. Diminution in urine flow, for example, was described by Porges and Pribram. Davis has observed antagonism of sodium chlorid diuresis by calcium in dogs. Besides this the elimination of injected saline fluids has, by Fleisher, Hoyt and Leo Loeb, been decreased by the intravenous injection of calcium chlorid.

The last named authors find, however, that calcium injection increases the tendency to peritoneal and pulmonary transudation. Augmented rather than reduced permeability would be indicated in such a case, unless one assumes that the calcium acts rather by hindering some normal reabsorptive process than by facilitating the escape of fluid into the affected cavities.

On the other hand, prevention of various experimental inflammatory edemas was accomplished by calcium injections in the hands of Chiari and Janusche.

In view of the present state of our knowledge it is not surprising that clinical applications of calcium in the treatment of effusions, coryza, etc., have been rather disappointing. The success attained by Choksy and others with magnesium sulphate in the reduction of the swellings of erysipelas and other inflammations is probably due largely to salt action.

Excess of calcium did not retard recovery from saline hydremia in the rabbits of Bogert, Mendel and Underhill, although a positive result might have been anticipated.

Body Temperature.—The effects of calcium upon the heat regulation have not been sufficiently investigated.

MacCallum, as mentioned above, describes an antipyretic effect from calcium in tetany and Hill has obtained a similar result in normal rabbits when small doses were administered intravenously. Five to eight c.c. of a five per cent solution of calcium lactate thus given cause an initial temperature fall of from 0.4° to 0.6° C. The higher of these doses produces toxic symptoms accompanying this temperature fall; a rise of from 1.5° to 2.5° C. then ensues, with disappearance of the other symptoms of poisoning.

Gum arabic (consisting largely of the calcium and magnesium salts of arabinic acid) when given in 7 to 20 per cent solution acts, temporarily at least, as an antipyretic agent in fevered rabbits and dogs, but not in healthy animals. In normal dogs, moreover, a considerable rise of temperature results. (Barbour and Baretz.)

Magnesium salts are stated by Schnetz(*b*) to reduce the body temperature even if the narcosis is prevented by calcium (as accomplished by

Meltzer and Auer). The latter fact might tend to exclude a centrally induced antipyretic action.

The prevention of sodium chlorid fever by proper concentrations of calcium salts (balanced solutions) has already been discussed.

In infants, Bosworth and Bowditch maintain that an excess of ingested calcium causes an accumulation of insoluble derivatives in the tissues. High temperature with toxic symptoms results and calcium lactate appears in the urine. The untoward effects are preventable by the administration of sufficient chlorid or phosphate to keep the calcium in soluble form.

Carbohydrate Metabolism.—The inhibitory effect of calcium upon sodium chlorid glycosuria has been discussed.

The effects of calcium upon blood and urine sugar in rabbits have been extensively investigated by Underhill(*h*). He maintains that calcium salts play a noteworthy rôle in the regulation of the blood sugar content; although lacking marked effect in normal animals they distinctly alter the character of the curve of epinephrin hyperglycemia, often augmenting the glycosuria. Furthermore, withdrawal of calcium (by administration of sodium phosphate or oxalate) produces *hypo*glycemia, curtailing the hyperglycemia and often the glycosuria produced by epinephrin. Underhill and Blatherwick showed that while thyreoparathyroidectomy results in hypoglycemia as well as in tetany, calcium lactate will temporarily restore the blood sugar to its normal level. These facts accord with the conception of tetany as an alkalosis.

After subcutaneous injections of magnesium sulphate Underhill(*j*) observed hyperglycemia and slight glycosuria when general anesthesia developed. With subanesthetic doses only a slight hyperglycemia, without glycosuria, was seen. Calcium antagonizes not only the magnesium anesthesia, but also the hyperglycemia. This would appear to classify the latter as of asphyxial origin, but Kleiner and Meltzer(*b*) have shown that it occurs under adequate artificial respiration.

Diabetics, according to Kahn and Kahn, exhibit a negative calcium balance. Following cautious injections of one-eighth molecular calcium chlorid into a vein these authors observed decreases in glycosuria, glycemia and polyuria. Relief of symptoms and prevention of acidosis were also attributed to the procedure. The renal factor appears to be largely responsible here and calcium therapy is unlikely to offer permanent relief, for with its employment no improvement in the capacity of the organism to oxidize dextrose has been demonstrated.

Brinkmann(*b*) has shown in frogs that an optimum calcium concentration is necessary to prevent the escape of glucose through the glomeruli. Jacoby and Rosenfeld's demonstration of the inhibitory effects of calcium upon phlorhizin diabetes [2] also indicates the significance of the renal factor.

[2] Retention of nitrogen and of acetone were also noted.

According to Salant and Wise calcium does not protect against zinc glycosuria in rabbits.

Upon the permeability of the kidneys for sugar, there appears to be no question of the inhibitory influence of the alkaline earths, but their excessive occurrence occasionally favors glycosuria, probably asphyxial in nature.

Purin Metabolism.—Abl maintains that calcium prevents cinchophen (atophan) from increasing the excretion of uric acid. But Gudzent, Maase and Zondek state that calcium, like cinchophen, increases the uric acid of the urine at the expense of the blood.

Pohl found that two grams of calcium chlorid per os decreased allantoin excretion from 0.397 to 0.104 gram. It did not alter the effect of epinephrin which was to increase both allantoin and uric acid excretion.

Strontium is stated by Lehnerdt to increase uric acid excretion.

Growth and Reproduction.—Emmerich and Loew found that the administration of calcium salts to female mice, guinea pigs and rabbits was followed by an increase in the number of pregnancies and of offspring. Pearl(*a*) has observed that such salts accelerate growth in female (but not in male) chicks and that this effect can be inhibited by corpus luteum extract. According to Cramer the growth in vitro of cells of mouse carcinoma is inhibited, with loss of water, by calcium chlorid. Sodium ions antagonize this effect.

Aluminium.—Schmidt and Hoagland maintain that aluminium, like calcium and magnesium, deflects phosphates from the intermediary metabolism in man. In special cases a low phosphate intake may be excreted entirely in the feces, in combination with aluminium.

III. Acids and Alkalies

Neutrality Regulation.—The mechanism which regulates the concentration of free hydrogen ions in the blood and tissues is very delicate. In sixty miscellaneous medical cases Levy, Rowntree and Marriott found the reaction of the serum normal ($P_h = 7.6\text{-}7.8$); the whole blood was also nearly unchanged ($P_h = 7.1\text{-}7.3$). Even when symptoms of acidosis are present the alkalinity is but little decreased (serum $P_h = 7.2\text{-}7.5$); alkali therapy combats this decrease. In diabetic coma Masel found $P_h = 7.11$ just before death.

The addition of hydrochloric acid to acidosis blood was found by Van Slyke to raise its H-ion concentration relatively more than when added to normal blood; thus the essential change in acidosis is loss of reserve alkali. VanSlyke defines acidosis as "a condition in which the concentration of bicarbonate in the blood is reduced below the normal level."

If the normal $\frac{H_2CO_3}{NaHCO_3}$ ratio $(=\frac{1}{20})$ remains undisturbed the condition is one of compensated acidosis, but should the respiratory center fail to remove the relatively excessive carbon dioxid present when bicarbonate has been lost the acidosis is said to be uncompensated.

Since excess of carbon dioxid gas in the blood may occasionally increase the numerator of the ratio without disturbing the denominator a true acidosis without change in the bicarbonate level is possible.

Next to carbonic acid and sodium bicarbonate the acid and alkaline phosphates of the corpuscles and tissues assist in maintaining the neutrality of the blood. The normal $\frac{NaH_2PO_4}{Na_2HPO_4}$ ratio in the blood plasma is given as $\frac{1}{5.1}$ by Michaelis and Garmendia.

Besides these defenses and the ammonia regulation (see "Acids"), a factor of possible significance in maintaining the neutrality is lactic acid. MacLeod and Knapp observed that this acid may appear in the urine, after alkali injections in animals, in amounts sufficient to account for five or six per cent of the alkali given.

Acids.—Walter in 1877 appears first to have shown that acids diminish the carbon dioxid content of the blood by displacing the "weaker" acid, H_2CO_3. Kraus and many others showed later that acids diminish the total or titratable alkalinity. Walter pointed out the differences between herbivora and carnivora with respect to their manner of regulating against acids. While the former to accomplish this must surrender their fixed alkali from the tissues,[3] the carnivora are able to deflect ammonia from the protein metabolism (at the expense of urea formation) for purposes of neutralization. Recently Loeffler has shown that acids inhibit somewhat the formation of urea by the perfusion of the liver *in vitro* with ammonium salts.

Thus an augmented $\frac{NH_3}{N}$ ratio in the urine has become a significant guide to acidosis.

The term "acidosis" may be understood in its broadest sense to include all those disturbances of the acid-base equilibrium in which there occurs either an actual increase in the P_h (i. e. in the $\frac{H_2CO_3}{NaHCO_3}$ ratio) of the blood, or, as is far more frequent, a decrease in the alkali reserve, or both. The appearance of the acetone bodies, as in diabetes, merely indicates one form of acidosis, sometimes designated as "ketosis."

L. J. Henderson and Palmer(*b*), as well as Hanzlik and Collins, have shown that acid sodium phosphate increases the urine acidity, although

[3] But Hart and Nelson have found a certain degree of ammonia regulation in cattle.

scarcely to an abnormal extent. The highest acidity figures in the two investigations were, respectively, $P_h = 5.3$ and 4.85.

Marriott and Howland(*b*) have found an interesting difference in the reaction of dogs to hydrochloric acid on the one hand and acid phosphate on the other. While the former increased the urinary ammonia parallel to the acidity, corresponding amounts of the latter gave, in spite of a great acidity increase, no change in the ammonia excretion. The authors attribute this to a difference in "strength" of the respective acids, "weak" acid being apparently unable to arouse the ammonia metabolism.

Alkalies. *Treatment of Acidosis.*—Walter established the efficiency of sodium carbonate injections in combating the acidosis produced by giving hydrochloric acid by mouth, even in the last stages. Using the alkali as a preventive a triple fatal dose of the acid could be withstood without increase in the ammonia excretion or the appearance of other symptoms.

In acid poisoning Salkowski and Munk and others have reduced the ammonia excretion to normal by giving fixed alkali.

In *diabetes* Stadelman(*a*) founded the theory of acid poisoning as the cause of coma and increased ammonia excretion, and instituted the alkaline treatment. Subsequently Magnus-Levy developed the use of alkalies by injection and per os, both in preventing and meeting the diabetic acidosis. The bicarbonate is now generally employed, its potential alkalinity being high in proportion to its actual (locally irritating) alkalinity. Even the subcutaneous injection, which may result in serious sloughing, may be accomplished with but slight irritation if the solution be first freed from all traces of the carbonate (Na_2CO_3) by saturating with carbon dioxid (Magnus-Levy).

The bicarbonate treatment should be instituted with the appearance of acetone substances in the urine; after the onset of coma it may be too late. The initial dose by mouth may be 30 to 40 grams in divided doses, freely diluted, given between meals. In coma oral administration may be supplemented by drop enemata (4 per cent), or, for a more prompt result, 1,000 c.c. of 4-6 per cent solution by vein.

In the acidosis of *anesthesia* Palmer and VanSlyke demonstrated depletion of the alkali reserve of the blood and suggested prophylactic injections of bicarbonate. Morriss employed this measure in gynecological cases (under chloroform or ether) and summarizes his results as follows:

C.C. OF CO_2 BOUND BY 100 C.C. OF PLASMA

	Before anesthesia	*After anesthesia*	*Difference*	*No. of cases*
Without bicarbonate	50.7	41.7	9.0	10
With bicarbonate	54.7	49.0	5.7	10

In studies of anesthesia Killian found the acidosis, increased diastatic activity and sugar content of the blood all controllable by alkali (e. g., 20-30 grams of bicarbonate per os). The blood acetone bodies in operative anesthesia Reimann and Bloom found increased sufficiently to account for from 20 to 100 per cent of the bicarbonate depletion. They endorse the recommendation that in cases where the carbon dioxid capacity is less than 58 c.c. the bicarbonate be used prophylactically.

The alkali depletion resulting from the overventilation usually accompanying light ether anesthesia can, as Henderson and Haggard have shown, be prevented by administration of a suitable carbon dioxid mixture with the anesthetic. Reimann and Hartman prefer the bicarbonate to the gas, believing it advisable to introduce more alkali into the body to combat the production of acid metabolites.

Uranium *nephritis* is associated, as MacNider(*a*) (*b*) has shown, with ketosis and depletion of the plasma bicarbonate. He finds that alkali injections protect against the toxic effects of uranium as well as against the unfavorable action which anesthetics exert upon the kidneys whether uranium-poisoned or "naturally nephropathic." Furthermore, the action of diuretics in these conditions is enhanced by sodium carbonate.

In the acute experimental nephritides of cantharadin, arsenic, diphtheria toxin and chromate poisoning Goto(*a*) (*b*) has reduced the acidosis with oral bicarbonate injections.

In the "retention acidosis" of nephritis Denis and Minot(*b*) find that small intermittent oral doses of bicarbonate keep the urine free of ammonia.

In infants a type of acidosis occurs during attacks of severe *diarrhea;* dyspnea is present but no cyanosis, and Czerny states that mineral acid poisoning in rabbits is simulated. Howland and Marriott(*c*) were the first to attempt the rescue of such children by the alkaline treatment. The blood was found free of acetone bodies in this condition. In one of their cases treated with bicarbonate the alveolar carbon dioxid tension (in millimeters) was on five successive days: 21, 42 ,54, 55, 41. The normal tension for infants is 36-45 millimeters. On the third day therefore the treatment was stopped.

Blood studies of such children have shown not only a depleted alkali reserve, but also a reduction from P_h =7.4 to P_h =7.2. Anuria is frequent and the acidosis is attributable to a retention of acid phosphate in the organism.

Schloss and Stetson have in similar cases reported, besides the decreased carbon dioxid in alveolar air and blood, a high ammonia coefficient and an increased "bicarbonate tolerance." 1.25-3.25 grams of sodium bicarbonate rendered the urine alkaline in normal infants, while 5.5-7.0 grams was required to accomplish this in cases of acidosis. Such

doses increased the carbon dioxid of the blood from 19.6-26 to 40-52 volumes per cent.

Water Metabolism.—Either acids or alkalies may act efficiently as diuretics. However, if the blood volume of rabbits has already been doubled by the intravenous injection of saline the addition of 0.4 per cent sodium carbonate does not hasten its return to normal. (Bogert, Mendel and Underhill.)

Alkalies enjoy considerable repute as obesity cures, Stadelman(*b*) and others having noted a marked reduction in weight during their prolonged use. Much of this may be attributed to water loss. (Digestive disturbances may, however, play a rôle.)

Bicarbonate edema sometimes occurs during the treatment of diabetes and other conditions with this alkali. Fitz associates it with a retention of sodium chlorid.

Body Temperature.—The relations existing between the acid-base equilibrium and the regulation of body temperature are not yet understood.

Mineral Metabolism.—A retention of intravenously injected chlorids (as well as of lactose) was observed by Herz and Goldberg after the administration of alkali. This was ascribed to renal action, and is confirmed by the observations of Fitz(*a*). On the other hand, Bunge and others have consistently observed an increased chlorid excretion after alkalies.

That acid administration per os increases the urinary calcium has been noted by Secchi as well as Givens(*a*) (*b*), in animals on a calcium-rich diet. Givens, however, found the calcium balance unaffected, and noted no appreciable increase in the magnesium excretion, in which two respects Secchi's work lacks confirmation. The latter found the sodium and potassium output after hydrochloric acid augmented for but a brief time, in contrast to the persistent ammonia excretion.

Stehle(*a*) found an increased calcium and magnesium excretion in dogs given hydrochloric acid by mouth. Sodium and potassium excretion were augmented to a lesser extent. He suggests a connection between calcium loss and diabetic acidosis.

Sawyer, Baumann and Stevens studied the mineral loss in children during acidosis and found both calcium and phosphates largely excreted. The loss of these ions varied with the severity of the acidosis.

Fitz, Alsberg and Henderson found that the administration of acids first increases the excretion of phosphates, but later this becomes diminished owing to exhaustion of the supply.

In experimental acute nephritis Goto(*b*) succeeded in diminishing the chlorid retention by oral administration of bicarbonate.

Total Metabolism.—While the effects of acid or alkali upon the total oxidations are not marked, there is some evidence that the former tends to diminish and the latter to augment the respiratory exchange. Chvostek

gave rabbits orally 0.9 gram (per kilo) doses of hydrochloric acid in 0.2 to 0.3 per cent solution. In four experiments both carbon dioxid output and oxygen absorption were reduced by about one-fourth, although decreased muscular activity was not noted. Lehmann obtained similar results under artificial respiration, noting also an increase in oxidations when alkali was administered.

Lactic acid causes a slight increase in the basal metabolism, as shown by Atkinson and Lusk.

Carbohydrate Metabolism.—The first evidence of a relation of the acid-base equilibrium to the carbohydrate metabolism was furnished by Pavy's discovery that phosphoric acid, orally or intravenously given, produces glycosuria in dogs.

Elias found that hyperglycemia accompanies acid glycosuria in dogs and rabbits. He and Kolb also showed that in the "hunger diabetes" of young dogs there is a diminution of the carbon dioxid of alveolar air and blood.

The inhibitory influence of alkali upon the glycosuria of ether and chloroform was discovered by Pavy and Godden, who abolished the sugar by the intravenous injection of sodium carbonate. In like manner Elias and Kolb inhibited "hunger diabetes."

Murlin and Kramer showed further that sodium carbonate introduced into the blood stream of a depancreatized dog lessens the sugar excretion. Bicarbonate was later found less effective. No compensatory increase of sugar was found in the blood and no evidence that the retained sugar is deposited as glycogen. The inference that alkali increases the combustion of sugar was only partially substantiated in such cases for, while in partially depancreatized dogs both mono- and disodium carbonate increased the respiratory quotient, the latter was found ineffective in cases where the entire pancreas had been removed.

Attempts were made by Murlin and Craver to treat human diabetes by the administration of alkalies through a duodenal tube. Sodium carbonate thus given reduced the glycosuria, but the bicarbonate curiously gave opposite results.

Underhill(*i*) showed that intravenous sodium carbonate usually induces a marked though transient fall in the blood sugar content of rabbits. He first suggested that the acid-base equilibrium is a factor in blood sugar regulation and showed further that both the hyperglycemia and glycosuria provoked by epinephrin can be prevented partially by sodium carbonate. He further pointed out the association between hypoglycemia and alkalosis in tetany and in hydrazin poisoning.

Applying the acid-base theory to therapeutics Underhill was able to maintain a diabetic individual in a state of comparatively good health and vigor over a period of years by giving large doses of sodium bicarbonate; as much as 120 grams was once given in a single day. The carbo-

hydrate tolerance in this case could be varied at will by appropriate changes in the dosage. (See figure 1.) On the other hand, Beard has been unable to control the sugar tolerance in this fashion. Fitz warns, in this connection, that the possibility of bicarbonate edema should be kept in mind.

The hyperglycemia resulting from etherization and operative procedure in sugar-fed dogs was reduced by MacLeod and Fulk by injecting

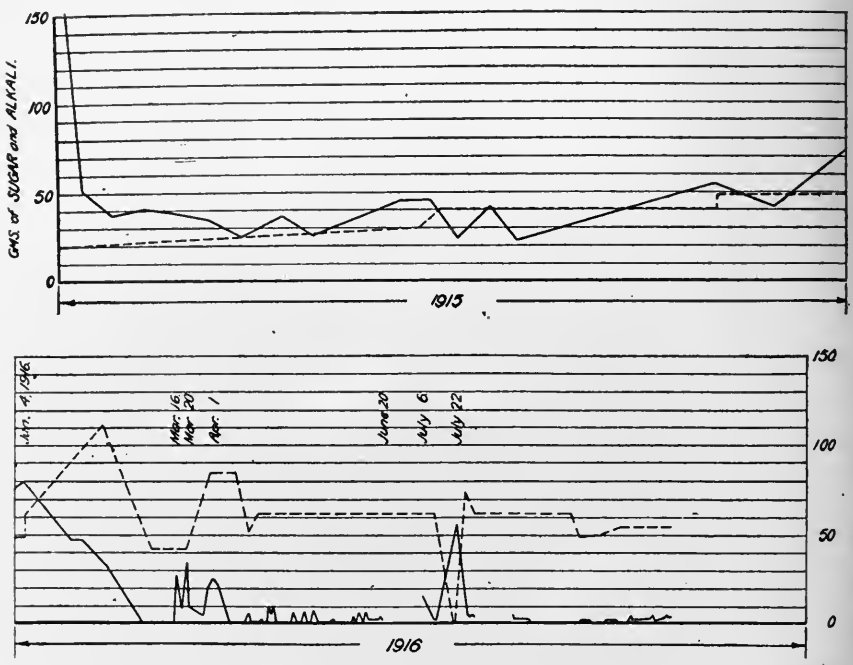

Fig. 1. Influence of sodium carbonate ingestion on the glycosuria of a diabetic: solid line, sugar; broken line, intake of sodium bicarbonate. (F. P. Underhill, J. Am. M. Assn., 1917, LXVIII.)

intravenously enough sodium carbonate to lower the P_h of the blood. (Compare Killian's results, mentioned above.) These investigators lay emphasis upon increased storage of glycogen in liver and muscles, under the influence of alkali.

The influence of alkali upon renal permeability for sugar was shown by the researches of Hamburger(*b*) upon the frog glomeruli. When the perfusion fluid contained NaCl, 0.6 per cent; $CaCl_2$, 0.0075 per cent; KCl, 0.01 per cent; $NaHCO_3$, 0.02 per cent and 0.1 per cent of glucose a "urine" containing 0.07 per cent of the latter was excreted, indicating a retention of 0.03 per cent. When, however, the bicarbonate content of the perfusion fluid was increased to 0.285 per cent, the equivalent of the normal frog serum content, a sugar-free "urine" was obtained.

While the exact effects upon either the combustion or the storage of glucose are not as clear as the influence of alkali upon renal permeability it may safely be affirmed that acids and alkalies tend, within certain limits, to increase and decrease, respectively, the excretion of sugar.

Protein Metabolism.—The augmented excretion of various protein metabolites, following administration of dilute mineral acids, described by some observers, is probably chiefly a diuretic effect. Alkalies have not been shown to affect appreciably the protein catabolism. Jawein found that 20-40 grams of sodium carbonate or citrate produced in man either inconstant changes or none at all. The neutral sulphur of the urine, however, appeared to be increased at the expense of the acid sulphates.

The retention both of non-protein and of urea nitrogen in the acute nephritis of metal poisoning, etc., was overcome in Goto's experiments by alkali administration.

The excretion of creatin in rabbits may be initiated or augmented by acids or diminished or abolished by alkalies, as shown by Underhill(*k*). Denis(*l*) and Minot(*d*) failed to establish such a relationship in a few human cases.

Purin Metabolism.—The alkalies have been extensively used in gout, partly on the theory that the supposed combustion increase would destroy more uric acid and partly in an attempt, by neutralizing this acid, to promote its excretion. We have seen, however, that increased oxidation has not been established and Ritter has shown that no direct solvent action of alkalies upon urate tophi can occur in the body. MacLeod and Haskins maintain that citrates by their alkalinity increase the elimination of endogenous uric acid and purins, but this may be due to intestinal derangement.

The "alkaline cures" for gout probably owe their beneficial effects merely to the considerable quantity of fluid ingested. In spite of the greater solubility of urates in alkaline form, alkalies do not remove gouty calculi from kidney or bladder; furthermore, alkalinity of the urine is likely to promote the deposition of phosphates.

Tetany.—Wilson and his associates found intravenous injections of hydrochloric acid effective in preventing the tetany which follows thyreoparathyroidectomy. They describe tetany as a condition of alkalosis. McCann found a lowered carbon dioxid capacity of the plasma in this condition and states that tetany may sometimes depend upon derangements in the acid-base relations of the alimentary secretions.

Harrop(*a*) has described a case of tetany resulting from the intravenous infusion of sodium carbonate in an adult suffering from mercuric bichlorid poisoning and totally anuric. He emphasizes the danger of the use of bicarbonate in cases of marked renal impairment. Tetany has occasionally been observed in young children given sodium bicarbonate for acidosis.

Boracic Acid and Borax.—Boracic acid and borax are respectively weakly acid and alkaline in reaction. Moderate doses of either do not effect the metabolism, but Chittenden and Gies(*b*) found that large quantities (5 to 10 grams per day for dogs) increase the urinary nitrogen; a dose of 4 to 8 grams in man retards the absorption of proteins and fats.

Under borax the body weight often falls, which has been attributed to augmented fat destruction by Rost and by Rubner(*i*), who found a corresponding increase in the carbon dioxid elimination. Boracic acid is said to be the least harmful of the food preservatives.

IV. Oxygen and Asphyxiants

Breathing undiluted oxygen produces no very significant effects, but when the supply of oxygen has been deficient asphyxial symptoms are promptly removed by inhalation. Lavoisier and Seguin in 1789 established the fact that pure oxygen under ordinary conditions does not affect the metabolism. Long continued exposure to atmospheres rich in oxygen produces pneumonia. (Karsner.)

Oxygen Deficiency.—Haldane has described the acute effects of atmospheres low in oxygen. Chronic oxygen-lack as seen in anemias, etc., causes considerable tissue destruction (Fränkel), fatty degeneration and acidosis, often with increased ammonia excretion. A. Loewy found amino-acids in the urine. Mansfeld attributes the increased protein metabolism to thyroid influence, for it fails to occur in the partial asphyxia of thyroidectomized dogs. In anemias with the hemoglobin as low as 20 per cent, Dubois has observed a marked augmentation of the total metabolism.

Exposure to rarified air, as first shown by Viault, increases the hemoglobin content. This is preceded by a *relative* hemoglobinemia (Dallwig, Kolls and Loevenhart). This blood concentration probably induces the fever of "mountain sickness" in which the temperature, according to Caspari and Loewy, sometimes attains 42° C. Such a temperature favors the free dissociation of oxygen, tiding over the period of preparation of better oxygen-transporting facilities. Douglas, Haldane, Henderson and Schneider at an elevation of 4,290 meters, found the hemoglobin sometimes increased to 150 per cent. Some evidence of "secretion" of oxygen into the pulmonary capillaries was found.

The total metabolism, in similar investigations by Wendt and by Dürig and Zünz was found increased, while there were evidences of a diminished protein catabolism.

Asphyxial Glycosuria.—Araki(*d*) and others have shown that simple asphyxia and other conditions associated with oxygen-lack cause an excretion in the urine of both glucose and lactic acid, the latter being regarded as a result of imperfect combustion. The glycosuria, like those produced

by piqûre and the emotions, appears to be of central origin. It cannot occur when the liver glycogen is exhausted. MacLeod has shown that, although it can still be produced with the liver denervated, it is preventable by double splanchnotomy, or excision of both adrenals. The effect is apparently due to increased hydrogen ion concentration of the blood (compare the acid glycosuria of Pavy) acting through the nervous centers, but involving often the coöperation of the adrenals.

Kellaway(*a*) (*b*) produced asphyxia by causing animals to breathe gas mixtures low in oxygen or high in carbon dioxid. Accelerated secretion of epinephrin and hyperglycemia were observed, both being due mainly to lack of oxygen, rather than to carbon dioxid excess. The hyperglycemia was only in part caused by acceleration of the epinephrin output. In anoxemia the ordinary mechanism of action is central, the splanchnics providing the path of the impulses.

F. M. Allen enumerates a list of poisons to which the production of asphyxial glycosuria has been attributed. Many of them will be discussed.

Blood Alkalinity.—Galleotti found in himself and several others as a result of several days' residence upon Monte Rosa (4,560 meters) a reduction of 40 per cent in the blood alkalinity.

Lactic Acid.—Araki's(*a*) finding of increased lactic acid excretion in conditions of oxygen-lack has been amply confirmed and so much stress was at one time laid upon this feature that, as Lusk points out, it was wrongly taken as pathognomonic of an asphyxial condition.

Terray found that when the oxygen percentage in the inspired air was reduced to 10.5 an increased respiratory activity commenced. With half of this concentration there was every indication of oxygen-lack, and the elimination of lactic acid became pronounced. The lactic acid eliminated as a result of breathing 3 per cent oxygen varied in eight observations from 1.206 to 3.686 grams in twenty-four hours.

Carbon Dioxid.—Carbon dioxid acts as a weak acid, serving as the respiratory regulating hormone. The central nervous system, especially the medulla, is so sensitive to its stimulating effect that it may become an important factor in the asphyxial phenomena just described. In high concentrations, however, the gas evokes the symptoms of oxygen-lack in the same way as when an indifferent gas such as hydrogen or nitrogen is inhaled; Loevenhart therefore refers its effects to interference with oxygenation. Westenryk showed that carbon dioxid inhalation reduces the temperature, Magyary-Kossa finding this effect more marked in fever than in health, and associated with reduced oxidations. To produce glycosuria 10 to 15 per cent of carbon dioxid (enough to narcotize) is required (Edie, Moore and Roaf).

Acapnia.—Excess of carbon dioxid is rapidly blown off by the respiratory mechanism and overcompensation often occurs, resulting in a lowered carbon dioxid content of the blood (Y. Henderson). Since this carbon

dioxid content runs essentially parallel to carbon dioxid capacity (i. e., varies with the alkali reserve of the blood), acapnia is a variety of acidosis.

Y. Henderson and Underhill showed that a lowered carbon dioxid content of the blood was associated after piqûre, pancreatectomy, light etherization, excessive artificial respiration and in other conditions with hyperglycemia and glycosuria.

Carbon Monoxid.—Clearly an asphyxial poison, carbon monoxid forms a firm combination with hemoglobin for which it has two hundred times the affinity of oxygen. When an atmosphere containing 0.05 per cent carbon monoxid is inhaled oxygen transportation is seriously hampered; 0.2 per cent is generally fatal, the hemoglobin then being about 60 per cent saturated with the poison (Haldane(*b*)). Carbon monoxid acts only by displacing oxygen, for when oxygen is breathed under two atmospheres pressure (which renders an animal independent of its hemoglobin) the addition of carbon monoxid in any amount produces no symptoms. Furthermore, in gas poisoning cases, oxygen if administered soon enough, which is rarely feasible, rapidly dispels the symptoms. Hemoglobin-free animals, for example, insects, exhibit no deleterious effects in the presence of carbon monoxid.

Blood Gases.—Saiki and Wakayama in carbon monoxid poisoning in rabbits found the carbon dioxid of the blood reduced from 30 to 5.21 volumes per cent; in dogs from 30-40 to 3.22 volumes per cent. The blood oxygen in the two species was reduced respectively from 12.64 to 7.62 per cent and from 20 to 2.01 per cent.

The low carbon dioxid content is not due to lessened carbon dioxid production, for, as Hans Meyer has shown, the latter must be very markedly reduced to produce even a slight diminution of the blood carbon dioxid content; it indicates rather a reduced alkalinity of the blood. Araki confirmed this by tritration and Saito and Katsuyama showed further an increase in the blood lactic acid in hens from 0.0269 to 0.1227 per cent. The fact that in dogs the blood carbon dioxid content is diminished so much more profoundly after carbon monoxid than after acid administration does not militate against acid production being the cause of this acapnia, for Loewy reminds us that acid feeding by mouth is one thing and acid formation in the tissues another; in the latter case, as, for example, in carbon monoxid poisoning, the fixed alkali becomes attacked before the ammonia regulation comes into play. Spiro, in fact, has demonstrated a marked acapnia as a result of the injection of acids intravenously (the ammonia regulation being thus more or less evaded). The occurrence of acidosis may satisfactorily be attributed to oxygen deficiency.

Total Metabolism.—Bock found in a dog subjected to an atmosphere of 0.2 per cent carbon monoxid (leaving less than half the hemoglobin saturated with oxygen) that the oxygen intake remained practically unchanged, while there was a considerable rise in the carbon dioxid excre-

tion. This result is often seen in oxygen-lack. Profound carbon monoxid poisoning leads, of course, to a diminished oxygen intake (Desplats), but in the grade induced by Bock it appears that the total metabolism remains unaltered. The high carbon dioxid output is attributable to displacement of the gas from the blood, first by the carbon monoxid itself; secondly by the decreased alkalinity as the condition progresses, and thirdly, probably temporarily by deeper ventilation.

Protein Metabolism.—An increase in the protein catabolism in man occurs, persisting for two or three days. Münzer and Palma found an increase in the phosphate excretion parallel to the nitrogen increase. In fasted dogs the nitrogen excretion is greater. Jeannert found 4.6 grams urea excreted in the 6½ hours following carbon monoxid poisoning as against 2.5 to 2.9 grams on control days. The increased catabolism is attributable to oxygen-lack.

The nitrogen partition, as has been observed, need not be altered in this type of acidosis; Münzer and Palma in man and Araki in animals noted only slight increases in ammonia excretion. Occasionally a very high uric acid excretion has been noted on the first day (Noel Paton). Fränkel failed to find amino-acids in the urine. Katsuyama and others find the synthesis of hippurates and of ethereal sulphates inhibited in carbon monoxid poisoning.

Mineral Metabolism.—Phosphate and sulphate excretion are probably increased, as in oxygen-lack. Kast found in carbon monoxid poisoning a decreased chlorid output in animals whose tissues were well supplied with this ion. In chlorid-poor animals, however, the output was increased. This apparent paradox is explainable upon the supposition that in the latter case an inherent tendency to lose chlorids is enhanced by the condition of oxygen-lack. The alkali-depleting mechanism is doubtless involved.

Lactic Acid.—Urinary lactic acid was found in carbon monoxid poisoning by Münzer and Palma and by Araki, blood lactic acid (in hens) by Saito and Katsuyama. Heffter found the acidity of the muscles of carbon monoxid-poisoned cats decreased. That the lactic acid appearance is due in part at least to reduced combustion accords with Araki's finding that subcutaneously injected lactic acid passes unchanged into the urine. If overproduction of lactic acid occurs in conditions of oxygen-lack, the experiments of Lusk and Mandel and others make it appear that this is derived from glucose, the glycogen of the liver being especially drawn upon.

Carbohydrate Metabolism.—Claude Bernard and Richardson gave the earliest accounts of carbon monoxid glycosuria. Araki showed that it is asphyxial. Straub(*a*) made the surprising observation that it is best obtained with meat feeding; after pure carbohydrate feeding carbon monoxid produces no glycosuria. The sugar is derived as in other asphyxial

glycosurias from the liver, and in the absence of liver glycogen none is excreted. Starkenstein has demonstrated the central mechanism of carbon monoxid glycosuria and claims by histological and chemical tests to have found the adrenal glands exhausted after carbon monoxid poisoning. In view of the work of Kellaway on asphyxial glycosuria, it seems probable that the central action is exerted through the nerves of the liver as well as of the adrenals.

Other Blood Poisons.—*Methemoglobinemia.*—A number of poisons besides carbon monoxid reduce the oxygen-transporting capacity of the blood. Among the poisons which do this by causing methemoglobinemia are the nitrates, chlorids, bile acids, pyrogallic acid, arsin, piperidin, toluylenediamin, hydroxylamin and others. Antipyretics, phosphorus and some heavy metals produce similar effects, but these constitute a minor part of their action.

When in its alkaline form, methemoglobin is much more readily converted back into oxygen. In accord with this, herbivorous animals appear less susceptible to its formation than the carnivorous. Alkali injections have therefore been suggested in the treatment of methemoglobinemia.

Acid-Base Equilibrium.—Diminished alkalinity of the blood was shown by Hans Meyer, Kraus, Kose and others to be commonly associated with the blood poisons.

Protein Metabolism.—Nitrogen excretion is increased by relatively small doses of chlorates (Mering(*a*)). Pyrogallol increases the excretion of nitrogen (Noel Paton), of uric acid (Künau) and of neutral sulphur (Bonanni(*a*)). Pyrodin (Fränkel(*b*)), toluylenediamin, and bile acids (Noel Paton), and large quantities of anilin, quinolin, salicylic acid, etc., also stimulate protein catabolism. Lawrence has shown that nitrites may increase the nitrogen and solids of the urine in man.

Benzol is a blood poison causing especially destructive changes in the hematopoietic organs, and diminution of the leukocytes and blood platelets. Increased excretion of neutral sulphur and of ammonia (Sohn) and a rise in body temperature also occur.

Carbohydrate Metabolism.—Hoffman observed glycosuria from amyl nitrite inhalation. This was associated by Konikoff with the disappearance of glycogen from the liver. Araki found the phenomenon associated with lactic acid secretion in both fed and fasted animals.

Hydrogen sulphid is one of the blood poisons that cause glycosuria (Cahn), but since sulphhemoglobin is found only in traces during life, E. Meyer believes the sulphid is directly toxic to the central nervous system. Other blood poisons causing glycosuria are the chlorates (Stokvis(*a*) and others) anilin (Brat), nitrobenzol (Magnus-Levy) and orthonitrophenol-propionic acid (Hoppe-Seyler).

Bukowski noted in phenol poisoning a rapid disappearance of liver

glycogen and Borchardt (cited by Allen) found glycosuria in rabbits after 0.5 c.c. subcutaneous injections.

Piperidin glycosuria was shown by Underhill to be accompanied by hyperglycemia and asphyxial in origin, disappearing under oxygen administration. Bühl and others produced glycosuria by inhalation of acetone, also an asphyxial poison.

Chlorid Excretion.—Kast found, as in carbon monoxid poisoning, an increased chlorid excretion after pyrogallol and toluylenediamin in chlorid-poor animals.

Syntheses.—Amyl nitrite inhibits ethereal sulphate synthesis (Katsuyama) and certain aromatic diamins which are also blood poisons were found by Pohl(*a*) to inhibit the synthesis of hippuric acid, but not of glycuronic or of ethyl-sulphuric acid.

Cyanids.—A type of asphyxial poisoning occurs in which neither the external respiratory mechanism nor the oxygen-transporting capacity of the blood is disturbed.

Claude Bernard pointed out that the venous blood in cyanid poisoning is red, although the other changes are those of asphyxia. He determined that the action of cyanid upon the blood is not the same as that of carbon monoxid since blood when mixed with cyanid will not turn red in the absence of air. In other words, the red color of the venous blood was ascribed simply to oxyhemoglobin. This was conclusively proven when Zeynek showed that at body temperature hemoglobin will not unite with cyanid, and oxyhemoglobin unites with it only after heating for several hours.

That the blood returns from the tissues still laden with oxygen was shown by Geppert(*b*), who obtained the following oxygen determinations in cyanid-poisoned rabbits:

VOLUMES PER CENT OXYGEN

Arterial blood	*Venous blood*	*Difference*
12.2	10.9	1.3
13.0	12.4	0.6

In various ways this investigator showed that the power of the blood to attach or to release oxygen is in no wise interfered with during cyanid poisoning.

Geppert showed further that the first effect of moderate doses of prussic acid upon the oxygen consumption of rabbits, cats, and dogs is one of augmentation, which is soon followed by a marked diminution. The return to normal in non-lethal poisoning is preceded by another wave of somewhat high oxygen intake. These stages are illustrated in the following table:

C.C. OXYGEN CONSUMPTION PER MINUTE

Animal	Normal	Poisoned 1st period	Poisoned 2d period	Return to normal	Normal
rabbit	22.7	....	15.8-17.4	.....	23.8
rabbit	20.7	....	5.0-9.4	.....	....
cat	35.4	40.2	21.2-19.8-24.8	.....	30.9
cat	30.9	60.4	24.0-28.9	44.8	....
cat	28.8	46.4	16.6-20.0	30.5-30.8	33.7
dog	39.7	80-52	26.1	60.6-53.2	39.3
dog	35.7	65-46	21.7	36.6-52.0	42.1

The "second period" presents the picture which is so characteristic of the toxic action of the cyanids. Now Geppert showed that this marked fall in the oxygen intake took place at a period when the ventilation was not reduced, but was enormously increased, i. e., the asphyxial demand for oxygen was present. Furthermore, the oxygen consumption was low not only during rest but during all stages of muscular restlessness up to actual spasms. During the convulsions, which often occurred, dogs occasionally (not always) exhibited an abnormally high oxygen consumption. In other species the oxygen intake was always subnormal even during the spasms. Similarly during the tetanizing respectively of normal and of poisoned animals Geppert found the oxygen consumption lower by two-thirds to four-fifths in the cyanid animals than in the controls.

The oxygen consumption was thus found reduced under circumstances in which an opposite effect would logically be expected.

The following are Geppert's figures for the carbon dioxid content of arterial and of venous blood:

C.C.CO_2 IN 100 C.C. BLOOD

No.	Normal Arterial	Normal Venous	Poisoned Arterial	Poisoned Venous	
34	41.1	....	22.0	48.2	Dog, art. at 1st spasm, venous during paralysis
35	43.7	....	18.0	....	Dog, moderate spasm
36	40.3	....	23.6	....	Rabbit, 6 min. after injection
33	....	50.3	17.7	....	Rabbit, after spasm
37	41.4	....	23.9	30.2	Rabbit, ven. at end of spasm, arterial during paralysis

No.	*Normal* Arterial	*Normal* Venous	*Poisoned* Arterial	*Poisoned* Venous	
29	....	....	....	35.3	Rabbit, ven. at beginning of poisoning, art. 4 minutes after spasm,
	....	....	7.7	17.0	ven. (2) 30 min. after spasm
38	36.0	46.2	11.0	33.1	Dog, severe paralysis
39	44.8	....	27.6	....	Rabbit, beginning of spasms

It will be seen that the carbon dioxid in the arterial blood was very low, often sinking rapidly (cf. No. 36); that of the poisoned venous blood was usually lower even than the carbon dioxid of normal arterial blood. A considerable degree of acidosis was therefore indicated.

This acidosis or acapnia, together with the increased ventilation (frequently the minute volume was more than doubled), was taken to account for the high respiratory quotients which occasionally exceeded 130, Geppert concluding that the actual production of carbon dioxid ran essentially parallel to the oxygen intake.

Since the return to the lungs of oxygen-laden blood was thus found associated with a profound depression of the oxidations Geppert depicted cyanid poisoning as "an internal asphyxia of the organs in the presence of superabundant oxygen."

This interference by cyanids with oxidation has been demonstrated under widely varying conditions throughout the realm of biology, e. g., in "salted" frogs (Oertmann), in excised kidneys (Vernon), and in many lower animals and plants. Hyman has shown a reversible decrease following a temporary increase (cf. Geppert's first period) of oxidations in sponges and presents an able review of certain theoretical aspects of cyanid poisoning. Child has shown that previous exposure to cyanids renders sponges more susceptible to oxygen-lack.

In hyperthyroidism it does not appear feasible to reduce the high total metabolism by cyanid treatment. (Snell, Ford and Rowntree.)

Ferments.—The (reversible) effects upon oxidative, hydrolytic (e. g., alcoholic fermentation of sugar) and other fermentative reactions are inhibitory (barring certain interesting exceptions). In Burge's experiments cyanid poisoning was found associated with a decreased blood catalase, but according to Duncker and Iodbauer the inhibitory concentration for catalase is not reached in acute cyanid poisoning.

Whatever may ultimately prove to be the exact nature of the cyanid-enzyme reaction in the tissues, Geppert's theory of "internal asphyxia" appears firmly established.

Body Temperature.—Increased heat elimination by blood dilution probably plays a rôle in the cyanid temperature fall of mammals (discovered by Hoppe-Seyler).

Carbohydrate Metabolism.—Zillessen describes an increased lactic acid excretion, but contrary to the results of some authors obtained no glycosuria.

Protein Metabolism.—Loewy finds that the total nitrogen excretion is notably increased (mainly as urea), and that amino-acid excretion occurs.

V. Phosphorus, Arsenic, Heavy Metals, Etc.

Phosphorus.—The effects of phosphorus upon the metabolism are associated with two distinct conditions, one largely of a catabolic nature, the other anabolic. To the first, the toxic syndrome, much attention has been devoted.

Phosphorus poisoning is characterized by profound liver injury, including fatty changes, in which respect the heart also is involved. The liver glycogen is soon exhausted. There are a wasteful excretion of nitrogen, a somewhat high total metabolism and an acidosis associated especially with a high blood and urine content in lactic acid.

While phosphorus was formerly assigned by many to the category of asphyxial poisons, Oswald and others have maintained that it acts chiefly by impairing the anti-autolytic agents of the body. The present-day theory of Lusk hinges largely upon the lactic acid accumulation.

Total Metabolism.—Phosphorus poisoning is not, as once believed, associated with a low level of bodily oxidations. Lusk has found that the oxygen consumption in this condition is augmented, which observation has been confirmed by Hirz. The former attributes the increase both to fever and to augmented protein destruction.

Fat Metabolism.—In spite of the obvious shifting of the bodily fat, its total combustion was found unaltered by Lusk. Loewi has compiled the figures of a number of observers with regard to fat and water content of the liver. The normal ether extract varied from 2.8 to 3.6 per cent of moist liver. The ether extract in phosphorus poisoning varied from 19.5 to 37.7 per cent of moist liver. The water content of the liver is slightly reduced when the fatty changes are marked.

With regard to the origin of the liver fat, Lebedeff showed that fat from other species injected subcutaneously in phosphorus-poisoned animals can later be identified in the liver. Furthermore, in such animals fat does not appear in the liver unless there is an ample store elsewhere in the body. The older hypothesis of true fatty degeneration (the fat being derived from the impaired cells of the affected organ) therefore became displaced by the theory of fatty infiltration. In support of this Taylor(*a*)

has shown in frogs that there is an actual loss of total body fat, that of the phosphorus-poisoned animals when killed being 22 per cent less than that of the controls. There was some increase in the gross weight of the poisoned frogs which Taylor ascribed to edema.

Shibata confirmed in mammals the diminution of total body fat after phosphorus.

Rosenfeld(*a*) (*b*) confirmed Lebedeff's results and found the blood content in fat increased under phosphorus, thus detecting the material in the stage of transportation to the liver. Leathes(*b*) showed that the liver alters the depot fats in certain respects, regarding this as a necessary preliminary to the utilization of the fats in metabolism. Fatty infiltration of the liver would represent an excessive attempt at such a conversion; it is found in all conditions in which there is a high need for fat (starvation, etc.). If such animals are freely fed, the fatty infiltration of the liver may disappear within a day (Mottram(*b*)). Rettig has shown that a carbohydrate-rich diet tends to prevent the fatty infiltration.

Carbohydrate Metabolism.—The finding by numerous of the earlier observers that glycogen soon disappears from the liver in phosphorus intoxication was confirmed by Welsch. Notwithstanding this, glycosuria is a comparatively rare feature; for example, Walko detected sugar in the urine of only 6 out of 141 patients. In these cases it was not associated with any special degree of severity or other definite feature. The blood sugar as Neubauer, as well as Frank and Isaak, found is, if anything, somewhat decreased. Thus phosphorus poisoning is differentiated from typical asphyxial conditions where glycogen disappearance is regularly associated with hyperglycemia and glycosuria.

Frank and Isaak regarded interference with the synthesis of glycogen as the primary action of phosphorus. They attributed the increased protein destruction to the need of compensation for a low energy production from carbohydrates.

The lactic acid which accumulates in phosphorus poisoning arises from glucose, as shown by Lusk and Mandel. For lactic acid disappears from the urine as soon as the phosphorus-poisoned dog is treated with phlorhizin; the glucose is hurried away before the lactic acid can be split off from it. In accord with this Fuerth has shown that the quantity of lactic acid elimination in phosphorus poisoning may be increased by feeding an excess of sugar.

Increased autolysis, especially in the liver, is regarded as the fundamental disturbance in phosphorus poisoning by Jacoby, as well as Porges and Pribram. The latter authors attribute this to oxygen deprivation. In this connection, Duncker and Iodbauer, as well as Burge, maintain that catalytic activity is somewhat decreased.

Ishikawa produced alimentary glycosuria early in phosphorus-poisoned rabbits but obtained no hyperglycemia, which fact he attributed to dam-

aged kidneys. He states that the glycolytic power of muscles and liver was low, that of the serum high.

Marshall and Rowntree demonstrated a decreased tolerance for galactose and levulose in phosphorus-poisoned dogs.

Protein Metabolism.—Storch first observed profoundly increased nitrogen excretion in phosphorus poisoning, finding a surplus of 200 per cent at times. Badt and others substantiated the increased catabolism. In fasting dogs poisoned by phosphorus, Lusk, Ray and MacDermott found the protein metabolism increased by from 83 to 183 per cent. They contrasted this gain with that obtained under phlorhizin which varied from 240 to 440 per cent. In the latter case, if phosphorus was given subsequently there was no further essential increase in protein metabolism. This was interpreted to mean that phlorhizin glycosuria is the predominating factor in such an experiment and that the anti-autolytic enzymes are inhibited rather by lactic acid than by the direct influence of phosphorus.

Lusk believes that "phosphorus may affect the conditions which lead to the oxidation of the lactic acid derived from glucose, and the accumulation of this acid may prevent the action of some of the deaminating enzymes; and further its non-combustion may necessitate an increase of protein metabolism."

Rettig has shown that a diet rich in carbohydrates prevents the increased protein catabolism. Simonds(*b*) advocates the use of a sugar diet in the treatment of phosphorus poisoning, not only as a source of energy, but also to inhibit abnormal enzyme action.

The anomalies of the protein metabolism in phosphorus poisoning include the appearance in the urine of amino-acids, especially leucin, tyrosin, cystin, and sometimes peptone-like substances. Gottlieb and Bondzynski, who first demonstrated that oxyproteic acid is a normal urinary constituent, found it increased in phosphorus poisoning. Mendel and Schneider found cynurenic acid increased. Wakeman has noted changes in the relative amounts in the liver of the basic amino-acids, histidin, arginin and lysin.

Lusk found the uric acid and creatinin excretion unchanged.

In Marshall and Rowntree's studies of the blood of phosphorus-poisoned dogs, non-protein nitrogen, urea, and amino-acids were all found increased. They noted a terminal acidosis.

Hauser showed that phosphorus inhibits the synthesis of hippurates.

Acid-Base Metabolism.—Hans Meyer and others have found the carbon dioxid content of the blood and the titration alkalinity markedly diminished. Besides the lactic acid, Meyer inculpates the sulphuric and phosphoric acids derived from protein.

Mineral Metabolism.—Welsch found the excretion of phosphates and sulphates increased, but that of chlorids diminished. Kast, however,

observed subsequent to the chlorid retention, an unusually high excretion of this ion.

Schloss(*b*) obtained negative results with phosphorus upon the calcium metabolism in rickets, but Brown, MacLachlan and Simpson find that phosphorus, especially in conjunction with cod liver oil, produces an increase in the blood calcium in tetany.

Phosphorus Deficiency.—Phosphorus deficiency leads to disturbances

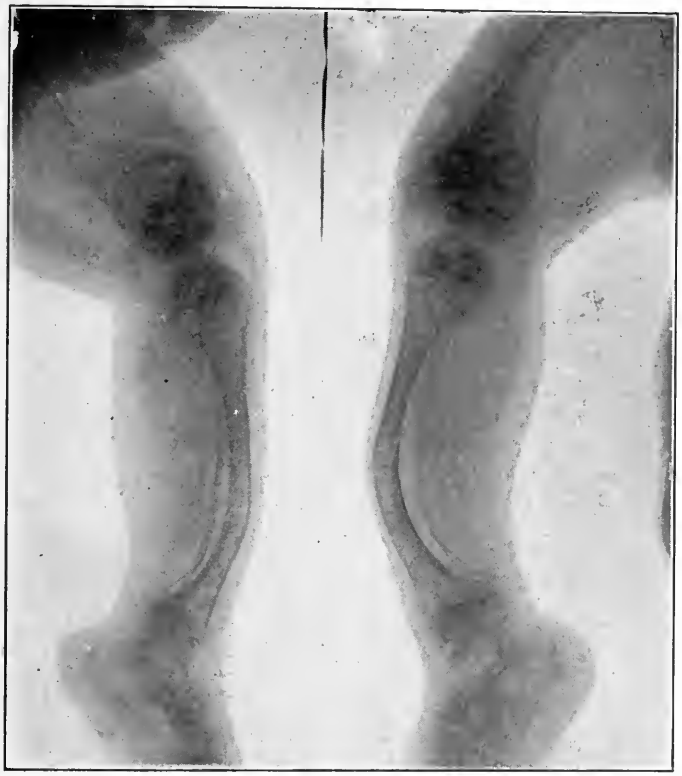

Fig. 2. Leg bones in osteogenesis imperfecta. Seven-year-old boy untreated. (D. B. Phemister, J. Am. M. Assn., 1918, LXX.)

in growth and nutrition, the bones becoming soft and flexible when their content in the element has fallen by about one-sixth (Heubner).

Effects upon the Skeleton.—Wegner in 1872 first demonstrated the favorable effects of phosphorus upon the formation of bone, thus bringing to light the anabolic aspect of phosphorus action. Small doses given to growing animals were found to result in a production of compact instead of spongy bone from the epiphyses. In adults the canals became filled with dense bone, having a normal structure and chemical constitution. Kassowitz found that larger doses increased the vascularization of the bone. He described favorable results from phosphorus in rickets, osteo-

malacia and delayed healing of fractures, establishing the therapeutic dose at 1 milligram daily with meals. Cod liver oil (10 milligrams phosphorus in 100 c.c.) is often used as a vehicle.

Jaw necrosis has been noted even with therapeutic doses. By laying bare the periosteum of the jaw and other bones in rabbits which were then exposed to phosphorus vapor Wegner showed that the necrosis is due to the direct action of the poison.

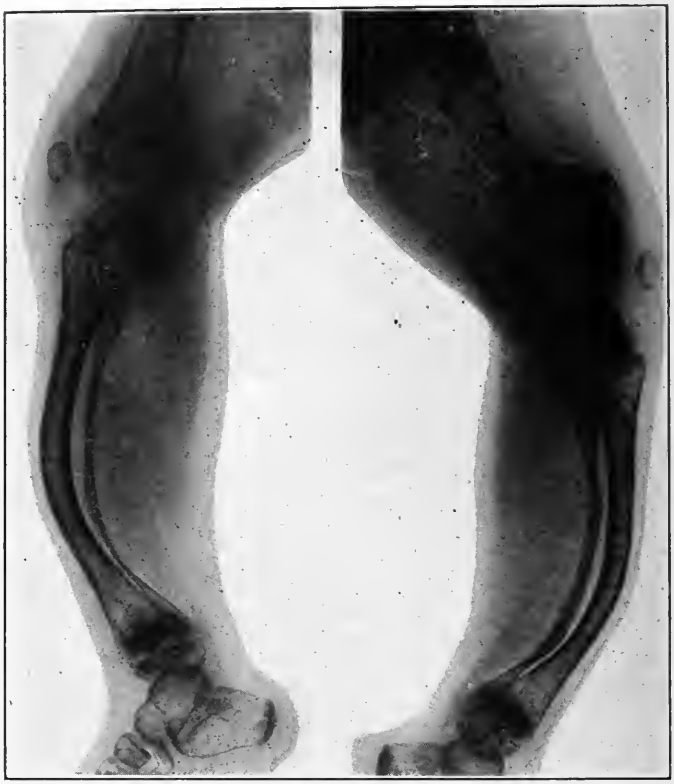

Fig. 3. Same case as Fig. 2 after two years of treatment with 1/150 grain phosphorus twice daily. (D. B. Phemister, J. Am. M. Assn., 1918, LXX.)

Definite effects of phosphorus upon the growth of normal and diseased bones in children have been shown by Phemister, employing the X-rays. Figures 2 and 3 illustrate the effects in the leg bones of a seven-year-old boy with osteogenesis imperfecta. Phemister administers 1/200 grain pills on an average of three times a day; the deposit of compact bone continues after the cessation of treatment.

Organic Phosphorus.—The alleged superiority of organic phosphorus compounds has not been substantiated; for example, Plimmer has shown not only that the body can synthesize its organic phosphorus from the

inorganic forms, but that the organic preparations themselves must undergo hydrolysis in the intestine whence they are assimilated as inorganic phosphates. On this subject reference should be made to the review by E. K. Marshall.

Lecithin was shown by Danilewski to hasten the growth of frogs' eggs and to augment assimilative processes in mammals. Cronheim and Müller produced with this phosphorus-containing lipoid a stimulating effect upon the protein anabolism.

Cod Liver Oil.—Cod liver oil was selected as a vehicle for phosphorus because for many years some unknown specific property as a nutritional stimulant had been ascribed to it, but more critical authors were inclined to regard it merely as a well assimilated food. Osborne and Mendel(*f*), however, have demonstrated a specific influence of cod liver oil upon the growth of white rats. Fats like lard, almond oil etc., do not possess this property which appears to be due to the fat-soluble vitamin. Schloss has apparently demonstrated for it a calcium-retaining power in rickets (see Calcium), in which disease Mellanby(*c*) finds it superior to all other fats.

Howland and Park recently have demonstrated the deposition of calcium in bone as a result of cod liver oil administration; in human beings this is demonstrable after three weeks. Marked increase in the blood phosphorus was also observed.

It seems probable, therefore, that cod liver oil promotes in some way the mobilization of phosphorus in the blood which in turn stimulates the calcium metabolism, perhaps through its peculiar tendency to augment the lactic acid content of the blood.

Hess(*c*) finds cod liver oil inferior to orange juice in the scurvy of guinea pigs.

Arsenic and Antimony.—With respect to its effect upon the metabolism, arsenic appears to occupy a position midway between phosphorus and the heavy metals. The stimulating effect upon bone formation, the fatty infiltration, the lactic acid excess, the loss of the capacity to store or to retain glycogen although glycosuria is rare, all bring it into close relationship with phosphorus. The fatty degenerative changes after arsenic are, however, less marked and the fat balance is positive. On the other hand, it appears to be a capillary poison, which fact is held to account for those profound intestinal disturbances which suggest the behavior of heavy metals.

The metabolic effects of antimony resemble those of arsenic.

T. Gies and others observed that repeated administration of small doses of arsenic to animals resulted in the production of a positive fat balance and new bone formation in which the long bones became thickened and the Haversion canals filled. That the therapeutic administration of arsenic improves the nutrition in a more subtle fashion than by merely stimulating the appetite or improving digestion is shown by the investiga-

tions, among others, of Henius(*a*). This author fed arsenic to dogs on a constant diet, observing increase in weight, a positive fat balance and stimulation of bone growth. The red blood cells and hemoglobin were also found increased under these conditions.

Total Metabolism.—The contribution of Henius includes perhaps the only investigation relating to the effects of therapeutic doses of arsenic upon the gaseous exchange in man. A chlorosis patient who was gaining weight under atoxyl was found to exhibit no difference in the basal metabolism as a result of the drug administration, but the experiments were not long extended.

Chittenden and Cummins gave rabbits 35 milligrams of arsenic daily and observed with these toxic doses some apparent diminution in the oxidations. Large doses of antimony gave similar results.

Nitrogen Metabolism.—When affected at all, the nitrogen excretion has usually been found increased by either arsenic or antimony.

After arsenic Boeck found no effect upon the nitrogen excretion in man, while Chittenden, Henius and others found an increase. With antimony Gaethgens(*a*)(*b*) found a 30 per cent increase in a fasted dog's nitrogen excretion. Chittenden and Blake, however, found the protein balance unaltered when 1-1.5 grams antimony oxid were given to a well-fed dog.

Arsphenamin induces metabolic effects similar to those produced by the inorganic arsenicals, according to Postojeff. Capelli found in syphilitic patients a high nitrogen loss on the first day after arsphenamin treatment, the only effect noted upon the metabolism. Sodium arsenate produced a nitrogen retention in two patients studied by Boyd. This may have been due to renal injury.

Uric Acid Excretion.—Abl found that arsenic and antimony in common with other intestinal irritants increase uric acid excretion.

Carbohydrate Metabolism.—Rosenbaum and others are agreed that arsenic induces a prompt disappearance of glycogen from the liver. The blood sugar content was not found increased, but work with newer methods appears called for. As with phosphorus, glycosuria at all events is rare. Saikowsky noticed that the arsenic or antimony liver becomes free of glycogen before the beginning of fatty infiltration can be detected. He was unable to produce glycosuria either by piqûre or by curare injections in arsenic-treated animals.

Konikoff showed that excess feeding of sugar did not restore the glycogen in arsenic poisoned animals. Luchsinger found that arsenic favors the production of alimentary glycosuria. Araki(*a*) found lactic acid, but rarely sugar in the urine in arsenic as well as in phosphorus poisoning.

Acid-Base Equilibrium.—Hans Meyer correspondingly observed a reduction in the alkalinity of the blood after toxic doses of arsenic. Mori-

shima, investigating the source of the lactic acid, noted that in autolysis of fresh livers the disappearance of glycogen is closely paralleled by the gains in lactic acid content.

Water Metabolism.—Arsenic, according to Magnus, exerts a specific toxic effect upon the endothelial cells of the capillaries throughout the body. To this the cholera-like diarrhea of arsenic has been ascribed. The dehydration is sufficient to cause marked thirst and to account for much of the hemoglobin increase. To this capillary effect Magnus also attributes the edema which sodium chlorid injections are capable of producing in arsenic-poisoned animals.

Karsner and Denis described in the glomeruli of the kidneys certain effects of arsenic which they associated with anuria. In their experiments nitrogen retention was rather slight, but caffein diuresis was frequent.

Body Temperature.—The well-known febrile reaction frequently following arsphenamin administration has been variously explained. It is not necessarily attributable to stale distilled water or to salt diuresis. Luithlen and Mucha have explained it as due to a destructive action of the drug upon the pathological tissues of syphilis. A new cause for some cases has been found in an alkaline-soluble substance extractable from new samples of so-called "pure gum" rubber tubing. (Stokes and Busman.)

Ferments.—Duncker and Iodbauer found an increased catalase action after small doses of arsenic, larger amounts giving negative results. This does not accord with the decrease after phosphorus. It must be borne in mind that catalytic activity of the blood has never been clearly shown to influence directly any vital process. Lacquer and Ettinger maintain that small doses of arsenic increase liver autolysis, which is retarded by large amounts.

Iron.—Stockman and Grieg have shown that five to ten milligrams of iron ingested per day suffice to maintain an equilibrium. The effects of iron deficiency are described by Hösslin(*a*). Organic iron compounds, whether or not the metal is readily ionizable, offer no real therapeutic advantage over the inorganic forms.

Like arsenic large doses of iron may cause renal and intestinal irritation with anuria and diarrhea. The carbon dioxid content of the blood is reduced with toxic doses (Hans Meyer).

Munk observed no changed in the nitrogen metabolism of dogs fed 0.3-0.5 gram daily.

Mercury.—The regular occurrence of nephritis and of glycosuria sharply differentiates the effects of mercury (as well as of uranium, etc.) from those of arsenic and phosphorus.

Certain effects common to the last two mentioned poisons are seen also after small doses of mercury, especially fat deposition and red blood cell increase. Schlesinger demonstrated these results in cats, dogs, and

hens fed for months on small quantities of corrosive sublimate. Among others Bieganski demonstrated similar effects in man.

Total Metabolism.—The total metabolism is not affected in fasting rabbits (Schroeder).

Protein Metabolism.—Böck and others found the nitrogen metabolism unaltered in syphilitics treated with mercury. Noel Paton observed a slightly increased nitrogen excretion in a dog. Urea and uric acid may also be increased after small doses. Schroeder and others have observed some nitrogen retention, presumably of nephritic origin, for the blood urea content is increased under such conditions.

Carbohydrate Metabolism.—Glycosuria was found by Schroeder and almost constantly by many others. Hyperglycemia was not found by Graf or Kissel in spite of the rapid disappearance of liver glycogen. Franck finally showed the glycosuria to be of renal origin. Lactic acid has not been demonstrated in the urine.

Fat Metabolism.—Fatty infiltration of various organs is frequently seen.

Mineral Metabolism.—Decalcification of bones with cachexia and anemia are typical of chronic poisoning.

Prévost, like others, found that mercury may produce calcium deposits in the kidneys, and associated them with a diminution in bone calcium.

Acid-Alkali Metabolism.—Hans Meyer first showed that the blood alkalinity may be diminished, and MacNider(*b*) found an acid intoxication in cases of delayed kidney injury.

Water Metabolism.—Jendrassik, the modern discoverer of calomel diuresis, recommended 0.2 gram doses four times a day. In cardiac dropsies seven to eight liters of urine were thus obtained daily with a considerable washing out of urea and chlorids.

Fleckseder(*b*) found that all mercury compounds by all methods of administration produce a diuretic effect in rabbits. He believes that mercury lessens the absorption of water from the small intestines; correspondingly larger amounts of water being absorbed from the colon, diuresis is more readily brought about. This does not explain calomel action in cardiac dropsies. The blood of rabbits becomes hydremic, but in man the hydremia seems to occur only with the dropsies. Healthy individuals under mercury may exhibit a concentrated blood associated with diarrhea.

Pleuritic exudates are not influenced by calomel.

Body Temperature.—Poisoning from inhalation of mercury vapor is accompanied by a febrile reaction (Carpenter and Benedict). Furthermore fever generally accompanies the stomatitis or skin eruptions of mercury poisoning, while in collapse there is of course a profound temperature fall.

Uranium.—In uranium intoxication while renal and capillary permeability appear to occupy the center of the picture, a kinship to phosphorus poisoning is still discernible. Edema, due to capillary poisoning, is often a feature.

Water Metabolism.—Léconte in 1854 described general anasarca and ascites as a result of the hypodermic administration of uranium acetate. Altered permeability of the capillaries was suggested by Richter as responsible for these changes. He found the edema not connected causally with salt retention. Fleckseder(*a*) excluded the renal factor, for he was able to produce the condition by giving uranium to nephrectomized animals, which do not develop hydrops without the poison. Further evidence of altered capillary permeability was furnished by Bogert, Mendel and Underhill, who showed that uranium interferes with the restoration of blood volume after large saline infusions.

Uranium poisoning is associated with various degrees of nephritis, and suppression of urine flow. In the earlier stages the oliguria may be partially overcome by caffein and the saline diuretics (Mosenthal and Schlayer). Diuretics do not, however, relieve complete uranium anuria, according to MacNider(*a*) who found the nephritis associated with an acid intoxication as evidenced by ketosis and a lowered alkali reserve. Inhibition of the nephritis with bicarbonate was found possible under some conditions.

MacNider found polyuria (accompanied by glycosuria) in the milder types of uranium poisoning.

Mineral Metabolism.—Pearce, Hill and Eisenbrey found a decreased chlorid excretion in uranium nephritis. Austin and Eisenbrey were later able to show that the smallest nephritic doses cause, along with the polyuria, some increase in the chlorids. Uranium (as well as chromates) may diminish chlorid excretion by 40 per cent for twenty-four hours.

Protein Metabolism.—The nitrogen excretion also ran parallel to diuresis or anuria in the experiments of Pearce and others, who confirmed the findings of Chittenden and Lambert that uranium increases protein catabolism, as shown by augmented nitrogen, sulphate and phosphate excretion. Mosenthal(*c*) found the non-protein blood nitrogen increased and pointed out that aside from renal retention this might be due to increase in the catabolism or to blood concentration. Karsner and Denis found the increase in non-protein nitrogen of the blood parallel to retention of phthalein.

Watanabe(*a*) finds in mild uranium nephritis that creatinin is less readily eliminated than urea; the opposite relation obtains in severe types.

Carbohydrate Metabolism.—Uranium glycosuria was discovered by Léconte and has been sometimes but not regularly found associated with hyperglycemia. Chittenden and Lambert found it dependent upon a sup-

ply of liver glycogen. Cartier associated it with intense degenerative changes in the liver. He failed to find lactic acid in the urine.

Fat Metabolism.—The degenerative changes in the liver in uranium intoxication have been associated by MacNider with acid poisoning. Fatty infiltration of various organs is common.

Total Metabolism and Temperature.—Chittenden and Lambert found the carbon dioxid output increased in uranium-poisoned dogs. This was associated with some increase in body temperature.

Chromates and Cantharidin.—The toxic effects of chromates as well as of cantharidin are said to resemble those of uranium. (Austin and Eisenbrey.)

Lead, Platinum, Copper, Zinc.—These metals are poorly absorbed and their effects upon the metabolism have received but little attention. (Loewi(*b*)).

Radium.—Gudzent maintains that the inhalation of radioactive emanations leads to an increased elimination of uric acid in the gouty, due to the conversion of the lactim form of uric acid into the lactam.

Contrary to these and other claims Fine and Chace(*a*) failed to produce any effect on the uric acid of the blood by radium given either intravenously or by inhalation. Berg and Welker state that radium salts given per os increase both nitrogen excretion and urine volume.

In chronic arthritis McCrudden and Sargent(*b*) could find no effect of radium water upon the excretion of uric acid, total nitrogen or water, although they state that the creatinin excretion may be affected. Recently, however, Theis and Bagg in the laboratory of S. R. Benedict have produced a marked increase in the uric acid excretion of Dalmatian hounds by intravenous injection of active deposit of radium.

Theis and Bagg found further that the active deposit of radium intravenously injected also increased the total nitrogen output, the urea curve running parallel; ammonia excretion was relatively as well as absolutely increased. Some increase in creatinin was noted after the increased temperature had returned to normal.

Variable results have been observed upon the respiratory metabolism, little effect having been demonstrated from the emanations. Benczúr and Fuchs(*b*) state that ingestion of 100 times the usual therapeutic dose of radium has caused a 17 per cent increase in the total metabolism. Alkaline radium water, on the other hand, is said to diminish the gas metabolism in health but not in gout. (Staehelin and Maase.)

According to Darms inhalation of radium causes a rise in body temperature followed by a fall, while a fall followed by a rise is seen after ingestion.

In the treatment of lymphatic leukemia Murphy, Means, and Aub found that radium affected the basal metabolism but slightly during the marked fall in the leukocyte count. In a similar case Knudson and Erdos

found under radium therapy very large increases in the excretion of total urea, ammonia, and phosphate, the latter sometimes attaining 400 per cent of the normal figure. The slight increase in uric acid excretion was attributed to the disintegration of nuclein tissue in the spleen.

Phlorhizin.—Although not used in therapeutics this poison is of great interest on account of the type of glycosuria it produces. Its effects upon the metabolism resemble somewhat those of the heavy metals.

Carbohydrate Metabolism.—Mering, the discoverer of phlorhizin glycosuria, found dextrose values in the urine as high as eighteen per cent; the absolute amount may be very large. The condition is characterized by absence of hyperglycemia, showing that it is essentially of renal origin. Zuntz showed that the effect upon the kidney was peripheral rather than central by injecting the poison into a single renal artery which gave rise to glycosuria at first on that side alone.

Although the important factor of increased glomerular permeability has recently been well demonstrated by Brinkmann(*a*) in Hamburger's laboratory some have deemed it necessary to seek further for the origin of such large amounts of sugar. Pavy, Brodie and Siau, for example, maintained that the kidneys form sugar from the proteins of the blood. Underhill, however, produced hyperglycemia by phlorhizin in animals in which the renal arteries were ligated, thus excluding the kidneys. Lepine(*b*) has long championed the "virtual sugar" theory in which much sugar is supposed to exist normally in combination with blood colloids, being demonstrable only on hydrolysis. From this source he believes sugar is derived in phlorhizin poisoning.

At all events the glycogen stores are never entirely exhausted by phlorhizin, even during fasting (Sansum and Woodyatt(*a*)). Epstein and Baer even maintain that phlorhizin stimulates glycogenesis, as hepatic glycogen seems to accumulate when the kidneys are excluded.

The sugar percentage in Brinkmann's perfusate being sometimes higher than in the perfusion fluid and no opportunity existing for reabsorption of water the renal secretory theory must still be given some consideration.

In complete phlorhizin poisoning Stiles and Lusk found that dextrose given subcutaneously fails to increase the respiratory quotient; thus the power to oxidise sugar becomes lost.

Protein Metabolism.—The body being deprived of the sparing influence of sugar there is often a very marked rise in the protein metabolism. Reilly, Nolan and Lusk have found this as high as 450 per cent of normal in dogs. After the extra sugar was flushed out the D:N ratio in this species was found to be 3.65 as against 2.8 in rabbits, cats, and goats. 58.7 per cent of the protein is therefore excreted as dextrose.

Fat Metabolism.—Mering in his experiments noted fatty infiltration of the liver when starving animals were phlorhizinized. This was asso-

ciated with increased ammonia excretion and ketosis. Moritz and Praus-nitz found that it could be prevented by carbohydrate feeding. Feeding butter fat or butyric acid will increase it. Bang(*i*) finds that, although the fat of the liver is increased, the blood fat remains unaltered.

Total Metabolism.—The heat production was found increased by Lusk, who attributes the change to the specific dynamic action of the increased protein metabolism. Recently Hári and Aszodi have observed a marked increase in the energy exchange and body temperature of starving dogs after subcutaneous injection of 0.05 gram per kilo of phlorhizin. Opposite effects were noted, with relatively larger doses, in rats. These authors believe that since the increased protein catabolism occurs in both cases it cannot be held to account for the increased heat production in dogs. They therefore postulate for phlorhizin a specific action upon the heat regulating centers.

VI. Narcotics

The *total metabolism* is reduced by all narcotic agents, whether classed as anesthetics or hypnotics, during the stages in which sleep is present. (For details see Jaquet.) This is the natural result of diminished muscular activity. The reverse may easily be demonstrated in the stage of excitement produced by some narcotic drugs.

The *body temperature* also has a tendency to fall during drug narcosis; as is well known this effect may result seriously if precautions to conserve bodily heat are not observed. Since anesthetized mammals also become more easily overheated than normal animals they may be described as poikilothermic. This has been attributed to inhibition of the regulatory influence of the "heat centers." (See Gottlieb, in Meyer and Gottlieb.)

Whether hydremia regularly results from the hyperglycemia and anuria which commonly accompany the action of all narcotic drugs is not known, but seems indicated from the reduction in hemoglobin described by DaCosta and Kalteyer. Hydremia would contribute toward a poikilothermic condition.

The narcotics will be further discussed under the following heads: General anesthetics, hypnotics, alcohol, opiates.

General Anesthetics. Chloroform and Ether.—*Protein Metabolism.*—The total nitrogen excretion is considerably increased both by ether and chloroform, as was first noted by Strassmann. Taniguti and others have found an increase in the chlorids and phosphates as well. Hawk and Kleine found an increase in neutral sulphur. Pringle found the nitrogen excretion diminished (renal effect?) during the anesthesia, but decidedly increased during the following twenty-four to forty-eight hours.

Hawk(*b*) found that the total nitrogen increase may amount to forty-five per cent. It is usually considerably smaller. Chloroform was espe-

cially studied by Howland and Richards and by Lindsay(*a*). The excretion of ammonia, allantoin, diamino-acids, polypeptids, creatinin and organic sulphur was found augmented; the urea and monamino-acids were decreased. Increased urea as well as total nitrogen and ammonia has been found by Aloi, however.

Rouzaud has recently reported interesting blood studies in surgical cases before and after chloroform. The average urea content of the blood was found increased from 0.048 per cent to 0.075 per cent. Under ether the blood urea was still higher. This investigator also noted an increased urea concentration in the urine.

Davis and Whipple have accomplished rapid reconstruction of liver cells in chloroform poisoning by feeding either carbohydrate or fat. In both cases the beneficial results were attributed to a sparing effect upon the protein metabolism.

Carbohydrate Metabolism.—Rosenbaum observed the rapid disappearance of glycogen from the liver under the influence of chloroform. Heinsberg found this effect associated with hyperglycemia.

Pflüger(*c*) states that glycosuria is comparatively rare after surgical anesthesia; Pavy and Godden prevented chloroform glycosuria by sodium carbonate. Hawk(*c*) described ether and chloroform glycosuria in dogs and found it more intense when the animals were well fed.

King and his pupils found that ether glycosuria is independent of the splanchnic nerves, but does not occur if the liver be excluded from the circulation. King, Moyle and Haupt proved that both hyperglycemia and glycosuria could be produced by intravenous injections of ether without causing asphyxia which was thus excluded from a primary causal relation. Ross and Hawk showed that ether glycosuria is not due to lowering of the body temperature.

Sansum and Woodyatt(*a*) made the interesting observation that both ether and nitrous oxid increase the glycosuria and D:N ratio in phlorhizin diabetes; the "extra sugar" is ascribed to glycogenolysis through tissue asphyxia. Ross and McGuigan observed a greater ether hyperglycemia in dogs on a pure meat diet than when carbohydrate was added. They obtained the phenomenon in the absence of asphyxia or excitement. The diastatic power of the serum was found unaltered. Watanabe(*b*) believes, however, that the blood diastases increase slightly just after the anesthesia.

Chloroform hyperglycemia was clearly shown by Scott to accompany the glycosuria. Marshall and Rowntree(*b*) have found that chloroform diminishes the tolerance to levulose and galactose as well as to dextrose.

Killian has found that patients under ether or chloroform exhibit an increase in both the sugar and diastase content of the blood, together with a decrease in the alkali reserve. All three of these tendencies can be reversed by the administration of 20-30 grams sodium bicarbonate.

According to recent work of Keeton and Ross ether hyperglycemia is

not prevented either by Eck fistula or the reversed operation; unilateral splanchnicotomy exercises some inhibiting influence, bilateral more. This appears largely due to an influence upon the adrenals which become implicated as in asphyxial glycosuria. Rouzaud found an average blood sugar content of 0.12 per cent in surgical chloroform anesthesia, ether giving a similar result.

Fat Metabolism.—Rosenfeld(*a*) (*b*) and others described fatty infiltration of liver, heart and kidneys after chloroform. The fatty and other changes of the liver have been extensively studied by Whipple and his pupils. This investigator ascribes to the hepatic lesions: icterus, disappearance of fibrinogen from the blood, diminution of liver lipase (with increase of plasma, kidney and muscle lipase) and the occasional excretion of leucin and tyrosin, as well as the other metabolic changes of chloroform poisoning. These claims appear well supported by the analogy to phosphorus poisoning.

That the blood fat is increased under ether more than any other anesthetic was maintained by Bloor(*c*), who found a rise of 40 to 100 per cent. Its water-solubility was considered the factor which favors ether in this regard. Berczeller gives 30 per cent as the maximum increase. Unless animals had been stuffed previously with fat food, chloroform was found ineffective until the second or third day when an "after rise" in blood fat occurred, which Bloor ascribed to the liver necrosis.

On the other hand, a lowering of the percentage of blood fat is described by Murlin and Riche; the intensity of this effect was found proportional to the degree of narcosis. Mann has found the cholesterol content of the blood unchanged under surgical ether.

Etherization of dogs for from one to one and a half hours on successive days has been found by Duceeschi(*a*) (*b*) to produce a marked increase in the cholesterol of the serum. This may persist for several days after the treatment. No untoward effects were noted in a twenty-five day experiment. Chloroform under similar conditions caused death within eleven days; the cholesterol remained high two or three days only, assuming a subnormal level thereafter.

Acid-Alkali Metabolism.—Marked increase in the titration acidity of the urine after long chloroform narcosis was described by Kast and Mester and others. Becker described acetonuria and pointed out the inadvisability of administering chloroform to diabetics. Thomas maintained that while the titration alkalinity of the blood was diminished the carbon dioxid content remained unaltered. This was ascribed to "carbon dioxid congestion," or insufficient ventilation. Abram described acetonuria after both choloroform and ether. Aloi recently found beta-oxybutyric acid in nine out of eleven cases of chloroform anesthesia.

Ether, chloroform, or nitrous oxide may reduce the P_h of the blood to 7.0 (neutrality), according to Menten and Crile.

Graham has made interesting studies of chloroform acidosis illustrating the protective effects of alkali. The diminished alkali reserve of the blood has been discussed in the section on alkalies.

Buckmaster has found the total gas content of the blood increased by 10.2 per cent under slight chloroform anesthesia. When the anesthesia was complete this was increased to 26.2 per cent. The extra gas is nearly all carbon dioxid, but there is also a low oxyhemoglobin content (40 per cent reduction).

Henderson and Haggard have made the important observation that the effects of ether upon the alkali reserve (as indicated by the carbon dioxid capacity) of the blood are dependent largely upon how the anesthetic affects the respiration. Ether in lower concentration, so administered as to cause hyperpnea, produces, acapnia, lowering the alkali reserve. On the other hand, concentrations of ether high enough to depress the respiration result in increasing the alkalinity of the blood. (Compare morphin.)

Water Metabolism.—Oliguria or anuria have long been recognized accompaniments of surgical anesthesia.

Rouzaud finds oliguria more pronounced with chloroform than with ether in man, in connection with his studies on hyperglycemia and azotemia. He recommends after-treatment with diuretics.

MacNider(*c*), however, has just reported some facts relating to anuria under anesthetics which would tend to discourage the use of diuretics and point rather to preventive measures. Dogs were anesthetized with ether, chloroform, or chloroform and alcohol (Gréhant's anesthetic). Ether anuria was found attributable to low blood pressure and rarely associated with depletion of the alkali reserve. Only in the latter case are diuretics ineffective. On the other hand, chloroform anuria (with or without alcohol) is invariably associated with loss of alkali, the kidney becoming quite impervious to diuretics.

Alkali preliminary to operative anesthesia is therefore recommended by MacNider from a new viewpoint—to protect the kidney.

Mineral Metabolism.—Kast found that chloroform, like some other poisons, increased the chlorid excretion more in chlorid-poor animals than in others.

Ferments.—Burge maintains that anesthetics lower the blood catalase content. Reimann and Becker found it increased in 35 per cent and decreased only in 65 per cent of their cases.

Hypnotics.—*Chloral.*—Mild chloroform action is suggested by many of the effects of chloral, although the former is not derived from the latter in vivo as Liebreich supposed. Chloral glycosuria was described by Eckhardt. Harnack and Remertz found that chloral increases both nitrogen and sulphur excretion, but later and to a lesser degree than does chloroform. Abl found an increased uric acid excretion.

Sollmann and Hatcher pointed out that severe chloral coma in animals is followed by anorexia, marasmus and loss of weight. They described the loss of heat-regulating power, Ginsberg the anuria and Winterstein(*b*) the decreased oxygen consumption. Cushny(*a*) describes a lowering of the carbon dioxid threshold for respiration after chloral and other hypnotics.

Amylene Hydrate diminishes the excretion of nitrogen, according to Peiser.

Sulphonal.—Stokvis identified the discoloration of the urine after sulphonal as due to hematoporphyrin.

Paraldehyd.—Powell states that "hypnotic" doses of paraldehyd lower the blood sugar in dogs without affecting the nitrogen excretion, while "anesthetic" doses increase the former and decrease the latter.

Urethan.—Chittenden observed that small doses of urethan decrease the nitrogen excretion, larger amounts having the opposite effect. Underhill(*c*) found that this hypnotic sensitizes rabbits to epinephrin glycosuria, while Bang(*e*) succeeded in producing hyperglycemia with large doses of urethan itself. This is stated to have been independent of the liver glycogen as well as of the adrenal secretion.

Alcohol.—As Atwater and Benedict(*a*) have shown, over 98 per cent of ingested alcohol is completely oxidized to carbon dioxid and water in the body. Its effects upon the metabolism are not extensive. The literature up to 1903 will be found reviewed in the report of Abel, Atwater, Billings, Bowditch, Chittenden and Welch.

Total Metabolism.—Reichert found the total metabolism in dogs unchanged by moderate doses of alcohol. In Higgins'(*b*) experiments on man the oxygen consumption was shown to remain unaltered after doses of 30-45 c.c. except in one-fifth of the cases; in these a slight increase was observed. Twenty to forty per cent of the total metabolism was due to combustion of alcohol. Large doses act like other narcotics in diminishing oxidations and paralyzing heat regulation.

Protein Metabolism.—Mendel and Hilditch in dogs and man found that, while moderate doses spare protein, loss of nitrogen occurs when large quantities of alcohol are administered. The partition of urinary nitrogen remains constant except that "toxic" doses result in an increased elimination of purins and of ammonia, accompanying other evidence of perverted metabolism, as indicated by the appearance of levorotatory compounds in the urine.

Salant and Hinkel observed in "subacute intoxication" in well-fed dogs a diminished excretion of total nitrogen and sulphur, a much greater decrease of inorganic sulphates and phosphates, and a tendency to chlorid retention. Neutral and ethereal sulphur were increased.

Carbohydrate Metabolism.—Allen has been unable to verify the claims of some authors that alcohol creates a diabetic tendency. Such was not

observed in cats and guinea pigs given either small or large quantities of alcohol for periods up to one week in duration.

In diabetics Benedict and Förök observed that replacement of fifty to eighty grams of food fat by isodynamic quantities of alcohol lessened the excretion of sugar, acetone and nitrogen. Higgins, Peabody and Fitz, however, could not prevent the appearance of acidosis in normal persons on a carbohydrate-free diet by giving alcohol. Mosenthal and Harrop found that the addition of alcohol to a carbohydrate-free diet does not alter the nitrogen balance in diabetes. No positive value in this condition has been demonstrated.

Fat Metabolism.—The fatty degeneration resulting from alcohol was described by Rosenfeld. Ducceschi found that repeated doses of alcohol sometimes tripled the total fat of the liver in association with an increase in its cholesterol and total solid content. The adrenals, on the other hand, lost forty per cent of their cholesterol, but gained slightly in total solids and fat.

Reproduction and Growth.—No effects of chronic alcoholism upon the offspring in man have been demonstrated as due to the poison itself. Stockard has observed the production of defective offspring in guinea pigs and other species, but Nice, on the other hand, finds in white mice that the offspring are normal and the growth of the alcoholic lines exceeds that of non-alcoholic descendants.

Opiates.—The opiates differ particularly from other narcotics in their tendency to increase rather than to reduce the alkali reserve and in the absence, in general, of changes in the fat metabolism.

Total Metabolism.—Various investigators have found the respiratory exchange reduced under morphin, but to this no unusual significance attaches since the reduction is essentially parallel to the narcotic effect. Higgins and Means, as well as Barbour, Maurer and von Glahn, have observed that sixteen milligrams of morphin sulphate will usually cause a definite depression of oxidations even when given after a fasting individual has been lying practically motionless for from one and a half to two hours. The latter group of investigators were able to diminish or prevent this effect by simultaneous administration of forty-milligram doses of tyramin hydrochlorid. Heroin (diacetyl morphin) in five-milligram doses does not appear to affect the metabolism (Higgins and Means), and the results of earlier observers with heroin and codein (Dreser) and other morphin derivatives appear to lack much positive significance.

Body Temperature.—The effects of morphin upon the heat-regulating mechanism were extensively studied by Reichert, who demonstrated that neither the depression nor the antagonistic pyretic effect of cocain could be produced after an operation interfering with the caudate nucleus of the corpus striatum. (For the effects upon total metabolism and body

temperature which are common to narcotics see the introduction to this chapter.)

Protein Metabolism.—Boeck found a six per cent diminution in urinary nitrogen in dogs, but Luzzato maintains that it is augmented by morphin in both fed and fasted animals, especially the latter.

Carbohydrate Metabolism.—Rapid disappearance of glycogen from the liver was noted by Rosenbaum and morphin glycosuria has been frequently described. Hyperglycemia and glycosuria were both found with large doses by Luzzato. The effects were not obtained in animals accustomed to morphin. Higgins and Means with therapeutic doses observed a very slight hyperglycemia and some decrease in the respiratory quotient. The latter seems attributable to the lowered ventilation.

Glycosuria may be simulated by the appearance of other reducing substances in the urine after morphin. (Spitta.)

Diabetes.—Good clinical observers claim that the glycosuria, together with thirst and polyuria, can be markedly diminished by the use of morphin. In this connection Klercker(*d*) has shown that, while opiates have no effect on hyperglycemia of hepatogenous origin, they may inhibit alimentary hyperglycemia. MacLeod suggests that this is due to retarded absorption induced by the depressant effect of morphin upon the alimentary canal.

Morphin, according to Kleiner and Meltzer(*a*), increases the renal elimination of intravenously injected dextrose, but retards the return of the blood sugar to its previous level, whence these investigators concluded that morphin increases the permeability of the kidney cells while decreasing the same kind of permeability of the capillary endothelia elsewhere in the body.

Ross(*a*) recently obtained marked hyperglycemia by the injection into dogs of 10 milligrams (per kilo) of morphin. In thirty minutes the blood sugar was increased by 59 per cent, in 45 minutes by 66 per cent, in one and one-half hours by 77 per cent. Ether administration begun one-half hour after morphin did not cause as much increase in the blood sugar as if morphin had not been used, but the final degree of ether hyperglycemia was the same with or without morphin.

Fat Metabolism.—Murlin and Riche found the blood fat decreased under morphin.

Acid-Alkali Metabolism.—Filehne and Kionka observed a diminution in blood oxygen but increased carbon dioxid after morphin. The latter is indicative of depression of the respiratory center which was first shown by Loewy to be less sensitive to carbon dioxid after morphin. The high carbon dioxid content of the blood is indicative of the presence of a greater alkali reserve.

The alkali reserve increase is proven by the increased alveolar carbon dioxid (shown by Higgins and Means, who observed the same under

heroin, and by Barbour, Maurer and von Glahn), the alkaline urine of dogs after morphin (Underhill, Blatherwick and Goldschmidt), and the increased carbon dioxid capacity of the blood after morphin (Henderson and Haggard, Hjort and Taylor). Henderson and Haggard interpret the phenomenon as illustrative of the power of the respiratory mechanism to exert an influence upon the alkali reserve of the blood. The extra alkali must be obtained, of course, at the expense of the tissues.

This effect of morphin is probably of value in the prophylaxis of operative acidosis (preventing acapnia with its consequent loss of blood alkali), but the bicarbonate prophylaxis possesses the advantage of furnishing new alkali to combat the acid production from various sources. The superiority of opiates over other narcotics may be related to their protecting effect upon the alkali of the blood.

Water Metabolism.—Ginsberg found that morphin decreases the urine flow in dogs, a property commonly exhibited by anesthetics. Opiates seem to promote the retention of water in the body by their action upon most of the secretions. The prevention of the exudation associated with colocynth diarrhea (Padtberg(*b*)) is pertinent in this connection. Furthermore, Bogert, Mendel and Underhill found the drug very potent in prolonging the retention of injected saline in the circulation. This hydremic tendency accords with its temperature-depressing capacity.

VII. Antipyretics

Antipyrin, Acetanilid, Phenacetin, the Salicylates, Quinin, Cinchophen (Atophan), and Related Substances.

In general the antipyretics resemble the narcotics in producing analgesia, anuria, hyperglycemia and increased protein metabolism. They differ from the last in failing to induce narcosis, glycosuria or fatty changes. Furthermore, given in therapeutic doses, they exhibit their hydremic, antipyretic and oxidation-depressing effects *only in pathological conditions associated with fever.* Significant changes in the acid-base metabolism have not been demonstrated in connection with their action.

Total Metabolism.—A large number of researches, involving the methods both of direct and indirect calorimetry, have been made upon the total metabolism and heat balance under antipyretic drugs. It may safely be regarded as established that antipyretic drugs, in man at least, do not act primarily by diminishing the total oxidations. Furthermore, marked increases in the heat elimination can be demonstrated.

In normal individuals so far as is known, therapeutic doses of none of the enumerated substances reduce the respiratory exchange at all. The quinin group, however, has occasionally been held to do this. In hitherto

unpublished work Barbour, Harris, and Plant have in normal fasting persons found the heat production increased in two experiments in which one-half gram was taken and practically unchanged in two others. These experiments accord with those of Zuntz as well as of Liepelt, who with large doses raised the total metabolism. Means and Aub found quinin of no value in reducing the basal metabolism in exophthalmic goiter.

With acetyl-salicylic acid in one gram doses there is produced in normal individuals approximately a six per cent increase (Barbour and Devenis). Wood and Reichert found the metabolism increased in dogs after large doses of sodium salicylate, which, according to Stühlinger, also increases it in guinea pigs.

Denis and Means found after repeated doses of sodium salicylate a fifteen per cent increase in the metabolism in one out of three surgical convalescents; the others exhibited no change.

With very large doses (two to three grams) of antipyrin Liepelt succeeded in producing a reduction in the oxygen intake varying from three to seven per cent. In the carbon dioxid output was found a greater diminution, probably attributable partly to retention. There was with these doses no significant temperature change. Even with antipyrin, however, there must often be an increase in the heat production. It usually raises the temperature, for example, in normal dogs and rabbits in doses which in fever are antipyretic; furthermore, it has a similar and more decided effect in decerebrate rabbits. This latter finding of Barbour and Deming was confirmed by Isenschmid, who also imitated it with sodium salicylate.

In fever the total metabolism is definitely depressed by therapeutic doses of the antipyretics, the natural result of cooling the body. With antipyrin Riethus observed reductions in the oxygen intake varying from two to thirty per cent.

After one gram doses of acetyl-salicylic acid Barbour observed an average diminution of 3.5 per cent in the heat production in association with a drop of nearly 1° C. in the temperature; heat elimination is greatly increased (see Fig. 4). Similar changes occur under phenacetin and antipyrin.

Quinin in fever has usually reduced the total oxidations in man and animals when the temperature was affected, for example, in a case of erysipelas studied by Riethus. Tuberculosis and many other febrile conditions respond to quinin by a rise in temperature and oxidations rather than by a fall. The contention that quinin, which is far from being a universal antipyretic, reduces temperature primarily by diminishing the heat production, certainly does not hold for human beings.

Senta found that various antipyretics reduce the oxidations in isolated muscles of mammals and birds, quinin and salicylic acid being the most efficient in this respect.

Protein Metabolism.—After antipyrin the nitrogen excretion is not much changed in man or in dogs. In fever it is often found reduced (Müller). This effect may, however, be simulated by renal retention.

Salicylates increase the elimination of nitrogen, as has been repeatedly demonstrated. Goodbody, for example, found urea and ammonia both increased. According to Wiley repeated ingestion of salicylate results in some loss of weight and of nitrogen.

Singer found both nitrogen and uric acid excretion increased after acetyl-salicylic acid in rabbits. Denis(*c*) and many others have found the uric acid excretion increased under salicylates. According to Fine

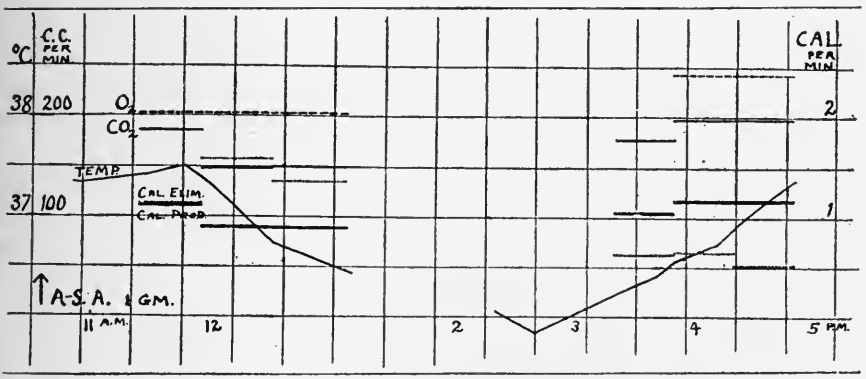

Fig. 4. Effects of acetyl salicylic acid on patient with tuberculous abscess; broken line, oxygen c.c. per minute; lighter horizontal line, carbon dioxid c.c. per minute; heavier horizontal line, calories produced per minute; dotted line, calories eliminated per minute; continuous curve, body temperature. Drug administered at arrow. (H. G. Barbour, Arch. Int. Med., 1919, XXIV.)

and Chace(*b*) this is due to increased permeability of the kidneys, for the blood uric acid is lowered.

Hanzlik has thoroughly reviewed the literature on salicylates. With Scott and Reycraft he demonstrated an accumulation of urea in the blood (associated with renal impairment and edema) after administration of full therapeutic doses of sodium salicylate.

Acetanilid in four to five gram doses increased the nitrogen metabolism of Kumagawa's dogs by over 30 per cent. Chittenden in normal men found the nitrogen excretion unaltered, but the urea was diminished by 10 to 20 per cent. Sulphates, phosphates, and chlorids were not significantly altered.

Quinin reduces the nitrogen metabolism definitely, as shown by Noorden and Zuntz and many others. Loewi found the percentage of urea nitrogen slightly decreased.

Reproduction and Growth.—Riddle and Anderson have shown that quinin fed to laying ring doves reduces the size of the eggs, the yolks

particularly being affected. They believe that the size attained is governed by restrictions placed upon the protein metabolism.

Carbohydrate Metabolism.—According to Lepine and Porteret and to Nebelthau antipyretics (antipyrin and acetanilid) are capable of promoting the storage of glycogen in both liver and muscles. Starkenstein's claim that antipyretics prevent the mobilization of liver glycogen by epinephrin has been disproved by Mansfield and Purjesz who found that antipyretics exert no demonstrable effect upon the somewhat variable curve of epinephrin hyperglycemia. Noorden examined the claim that salicylates decrease the sugar output in diabetes and failed to establish it.

Herter (cited by Underhill) observed the production of glycosuria after painting salicylate upon the pancreas of a dog. No other case of glycosuria due to any of this group of drugs appears to have been reported. Wacker and Poly have, however, described a rise in the blood sugar content in rabbits and tuberculosis patients after phenacetin and Silberstein found hyperglycemia after giving quinin to dogs.

Barbour and Herrmann demonstrated that hyperglycemia (without glycosuria) occurs in both normal and "coli fever" dogs after acetylsalicylic acid, sodium salicylate, antipyrin and quinin. The following averages were obtained:

DEXTROSE CONCENTRATION IN BLOOD

	Before Antipyretics	*Maximum After Antipyretics*
	%	%
Average of 13 normal dogs	0.137	0.186
Average of 10 fevered dogs	0.139	0.218

Since the blood of the normal dogs became slightly concentrated and that of the fever dogs diluted by the various drugs the absolute increase in the blood sugar content of the latter was somewhat larger than would appear from the concentration.

Antipyretic drugs cause no significant changes in the respiratory quotient.

Water Metabolism.—Barbour and Herrmann found after antipyretics a hydremia, as indicated by the hemoglobin content, in "coli fever" but not in normal dogs, as has just been stated. This is induced, at least in part, by the osmotic action of the extra blood sugar. The reason that the hydremia is not seen in the normal dogs appears to be that fever dogs are possessed of a store of available water in the tissues which is not normally present. This contention is supported by Barbour and Howard's demonstration of an increase in the percentage of blood solids during the initial rise of "coli fever," without diuresis. Furthermore, water would be liberated with the increased protein catabolism of fever.

In Hanzlik's demonstration of salicyl anuria one sees a further reason why the hyperglycemia tends to keep the volume of the blood high.

Hirschfeld maintains that antipyretics relieve diabetes insipidus and Gaulier finds that salicylates diminish the excretion of chlorids. These and various other observations tend to support the belief that salicylates induce oliguria.

In Hanzlik's non-febrile cases the hemoglobin remained constant. Barbour has found the hemoglobin percentage diminished in fever patients during the antipyretic action of both acetyl-salicylic acid and antipyrin.

The rôle of the excess sugar in producing hydremia is illustrated in Barbour and Howard's results with dextrose in normal and fever dogs. Intravenous dextrose injections, which in normal animals produce a slight blood dilution with no temperature change, will dilute the blood two or three times more extensively in fever animals coincidently with a marked antipyretic action. These effects are short-lived when much sugar is used. The sugar runs off in the urine presently and may leave the blood more concentrated and the temperature higher than ever.

Theory of the Mechanism of Fever Reduction by Drugs.—All antipyretics act by increasing the heat elimination; reduction in heat production is incidental. Antipyretics increase the blood sugar concentration. In fever extra water being available in the tissues, these drugs produce plethora; factors other than hyperglycemia may contribute to this result. Plethora promotes the dissipation of heat by radiation and surface evaporation. (Sweating is not essential to antipyretic action which proceeds unabated in the presence of atropin antidiaphoresis.) In health no plethora occurs,—consequently there is no antipyretic effect.

The earlier work on the relation of *"heat centers"* to antipyretic action is well presented by Gottlieb in Meyer and Gottlieb's pharmacological treatise.

Barbour and Wing have showed that local applications of antipyrin, chloral or quinin to the heat centers in rabbits all gave better antipyretic effects than the same doses by the intravenous or subcutaneous routes.

Hashimoto later found that the antipyretic action of both antipyrin and salicylate is enhanced by heating the centers but annulled by cooling. After quinin only heat was found effective, cold having no effect. The effects of heat and cold were prevented by morphin, as indeed the present author has often noticed to be true of ether.

Vasomotor effects figure largely in these "heat center" reactions which it is expected can be correlated ultimately with the blood dilution theory.

Acid-Alkali Metabolism.—Meyer found no change in the alkalinity of the blood with salicylates. In fatal poisoning, however, Walter found a low carbon dioxid content. Acetonuria is reported by Langmead and by Lees from large doses of salicylates, and in children. Piccini found that phenacetin and acetanilid, and, to a lesser extent, antipyrin, reduced

the arterial oxygen in dogs, the carbon dioxid being reduced to a slight extent. In general then the tendency is toward the side of acidosis.

Quinin and its congeners.—Although it is not a dependable antipyretic in many instances, Solis Cohen has recommended the use of quinin in pneumonia; the initial dose is 1-1.6 grams of the quinin-urea hydrochlorid, to be followed by 1 gram doses every three hours until the temperature is reduced to 102° F., which may require a day or two. Cahn-Bronner maintains that in certain lung inflammations treated with 0.5 gram doses of quinin subcutaneously an early antipyretic effect was seen and the mortality reduced to one-fourth. It may be of some real etiotropic value in this condition.

In malaria the drug only prevents "chills" and further symptoms rather than modifying the temperature curve after it has begun to rise. Certainly it does not compare favorably with other antipyretics in mild fever. Quinin is probably only antipyretic in nearly or quite toxic doses, when it acts very similarly to other types of antipyretic drugs.

Ethylhydrocuprein has a lesser antipyretic effect than quinin, as shown by Smith and Fantus.

Cinchophen (Atophan).—Cinchophen, according to Starkenstein and Wiechowski, reduces the temperature of normal rabbits by several degrees. Its real therapeutic value perhaps lies more in its analgesic properties (which it shares with other antipyretics) than in its influence upon the purin metabolism. For example, a number of compounds chemically related to cinchophen, but possessing no influence upon uric acid excretion were found by Klemperer to diminish in time and intensity the inflammatory phenomena of acute gout attacks. Boeck as well as Rotter has described the action of a number of other derivatives.

Purin Metabolism.—Nicolaier and Dohrn introduced cinchophen for the treatment of gout, having noted that three grams given daily to normal individuals increased the uric acid excretion sometimes up to 200 per cent of the normal. (6-gram doses tripled the output.) The increased excretion begins within an hour, the maximum being reached within two hours (Griesbach and Samson). The uric acid concentration shows, according to Haskins(*b*) (*c*), a compensatory decrease, sometimes during administration.

The increase of uric acid is often so great that it precipitates in the urine before it is passed. Haskins has in fact shown that cinchophen interferes with the urate-solvent action of the urine.

Zuelzer(*b*) maintains that the urate excretion is more prolonged in gout than in health.

Among the theories advanced to account for the action of cinchophen are increased destruction of nucleo-protein (Schittenhelm and Ullman) and conversion of absorbed uric acid into a filterable form (Frank and Pietrulla). Since, however, Folin and Lyman(*b*) were able to show a

decrease of blood uric acid parallel to the urinary uric acid increase, little need is found for an explanation beyond that of increased permeability of the kidney for this metabolite. According to McLester the blood uric acid eventually attains an irreducible minimum.

Fine and Chace have shown that when the administration of the drug is stopped the initial blood concentration is restored in from two to four days.

According to Starkenstein and Wiechowski the allantoin excretion is reduced and the total formation of purin bodies is inhibited. The same authors maintain that piqûre and asphyxial glycosurias are inhibited as by calcium, and that the drug besides an antipyretic possesses an antiphlogistic action, entirely inhibiting mustard oil chemosis.

VIII. Ammonia, Amins, Alkaloids, Purins, Etc.

Ammonia.—Underhill and Goldschmidt showed that organic ammonium salts are quickly and completely transformed into urea. The fate of the inorganic salts is more complicated. While a part are converted into urea another portion is excreted unchanged. Still a third part of the inorganic salts are temporarily retained, following which an augmented nitrogen excretion is noted.

Grafe found that ammonium salts increase oxidations in rabbits.

Hydrazin.—Underhill and Kleiner(*a*) showed that this poison produces fatty degeneration of the liver. Underhill and Murlin showed that it increases the respiratory quotient of fasting dogs, the increased combustion of sugar accounting for the hypoglycemia which occurs. It does not specifically affect the heat production.

Ethylenediamin.—This proteinogenous amin lowers the body temperature of rabbits: a tolerance to this effect is acquired within a few days. (Barbour and Hjort.)

Iso-amylamin, Phenylethylamin, and Tyramin.—All of these increase the nitrogen metabolism, especially in thyroidectomized animals (Abelin). Tyramin increases the total metabolism in man, lowering the alveolar carbon dioxid, as shown by Barbour, Maurer and von Glahn. These effects are antagonistic to morphin action. Phenylethylamin and tyramin raise the body temperature of dogs. Morita found that tyramin and similar drugs cause glycosuria, and Iwao that tryamin produces hemosiderosis in rabbits.

Beta-tetrahydronaphthylamin.—This is the most powerful pyretic poison known. Mutsch and Pembrey have shown that it increases the carbon dioxid excretion but not that of nitrogen. DeCorral maintains that it causes hyperglycemia and increases the hyperglycemia caused by narcotics.

The Amino Acids.—Increase of the total metabolism and body temperature (Lusk(*e*)), also the uric acid metabolism, by the amino acids has been well established (Lewis and Doisy).

Atropin, Pilocarpin, etc.—*Total Metabolism.*—Edsall and Means as well as Higgins and Means found the respiratory exchange increased after milligram doses of atropin in human subjects. On the other hand, Keleman, employing large doses in dogs, finds a decrease in the carbon dioxid output. This antagonizes the ten per cent increase in the metabolism which he has found after pilocarpin, confirming the observations of Frank and Voit(*b*). The relative rôle of secretory and smooth muscle activity has been discussed by Loewi. An energetic pilocarpin sialorrhea may deplete the blood fluid sufficiently to cause a rise of temperature with consequent increase in the total metabolism.

Protein Metabolism.—Either fifteen milligrams of pilocarpin or ten milligrams of atropin increased the nitrogen excretion in Eichelberg's experiments. There was a slight phosphate increase as well. With *scopolamin* de Stella observed in two rabbits and two dogs a consistent fall in nitrogen, chlorids, phosphates, and water in the urine. Uremia has been described in *muscarin* poisoning by Clark, Marshall and Rowntree, who found it due to renal impairment.

Purin Metabolism.—Abl found that atropin prevents the uric acid increase after cinchophen; Mendel and Stehle found the postprandial uric acid increase inhibited by the same drug.

Carbohydrate Metabolism.—Raphael and others have described glycosuria in atropin poisoning. Pitini, as well as MacGuigan(*a*), has observed that large doses increase the blood sugar. The conception was at one time prevalent that atropin was of value in the treatment of diabetes and in fact that it inhibited glycogenolysis. Mosenthal(*b*) has shown that the view that atropin increases the tolerance for sugar is unsupported by valid evidence.

Ross(*b*) finds that atropin reduces markedly the ether hyperglycemia, for example, from a forty-one per cent increase to a nine per cent increase in the first fifteen minutes, and from a fifty-seven per cent increase to a twenty-one per cent increase in the first hour. Atropin alone did not affect the blood sugar content.

According to MacGuigan pilocarpin may cause a delayed reduction in the blood sugar content. In massive doses atropin fails to lessen the hyperglycemia due to stimulation of the celiac plexus.

Mushroom (*muscarin*) poisoning may provoke renal glycosuria, according to Alexander.

Water Metabolism.—Pilocarpin has no direct action upon the urine (J. B. MacCallum), but owing to the great loss of fluid by other channels Asher and Bruck state that it usually diminishes the water and chlorids.

Cow has shown that a number of supposed effects of these drugs upon the renal function simply arise from actions upon the ureteral musculature.

After repeated injections of large doses of pilocarpin Waterman observed both diuresis and glycosuria, attributing these to increased renal permeability.

It is not unusual for three liters of sweat to be removed by pilocarpin diaphoresis, thus eliminating 2.5 grams of nitrogen. In nephritis this could amount to eight grams, thus affording notable relief for the kidney. (Sollmann.)

Body Temperature.—Both pilocarpin and atropin may cause hyperthermia, the former by secretory (especially salivary) dehydration and smooth muscle and gland stimulation (Reichert), the latter by central stimulation, perhaps associated with depression of the sweat. Atropin does not, however, hinder the action of antipyretic drugs.

Strychnin.—This alkaloid may be classed as an asphyxial poison for the reason that such effects as it exerts upon the metabolism are, in part at least, due to oxygen-lack. In view, however, of its most characteristic action being a direct stimulation of the central nervous system it is natural to invoke this stimulation in explanation of the glycogen discharge which strychnin produces.

Carbohydrate Metabolism.—The knowledge of hepatic glycogenolysis and glycosuria as a result of strychnin poisoning dates back to the work of Schiff (1859). Zuntz made use of the drug to demonstrate the formation of glucose from the protein metabolism. After ridding a rabbit of glycogen by strychnin convulsions he kept the animal fasting and chloralized for one hundred and nineteen hours. During this time 5.25 grams of sugar were excreted in the urine, and yet 1.286 grams of glycogen were still found in the liver and muscles. This must have arisen from protein.

Araki observed that strychnin causes lactic acid as well as glucose to appear in the urine, and classified it as an asphyxial poison, as did Starkenstein.

Lepine(*a*) states that strychnin glycosuria is unknown in man.

According to Blum strychnin is able to free the liver of glycogen if either both vagi or both splanchnic nerves are cut. He concludes, therefore, that glycogenolysis resulting from excessive muscular work is brought about through the blood.

Lusk has shown that strychnin and other convulsions cause the appearance of lactic acid in the blood, to which phenomenon, however, an adequate glycogen store is essential.

The alveolar carbon dioxid tension is unaltered by strychnin in therapeutic doses (up to 4.5 milligrams) in man, according to the results of Higgins and Means. These investigators, as well as Edsall and Means,

were also unable to produce any change in the total metabolism by such doses.

Some Other Convulsants—Camphor.—Edsall and Means, also Higgins and Means, have observed a slight increase in the total metabolism in man after 0.4-0.5 gram subcutaneous injections of camphor. The only change observed by these investigators in the alveolar carbon dioxid tension was a slight diminution in one case. This accords with Wieland's finding that camphor lowers the respiratory threshold for carbon dioxid in rabbits. The latter observed a similar result from coriamyrtin (a picrotoxin-like convulsant).

Since camphor is excreted in the urine in combination with glycuronic acid (Schmiedeberg and Hans Meyer) it is of some importance that this defensive mechanism should be intact when the drug is administered in large amounts; its toxicity is said to be higher when glycuronic acid formation is disturbed through starvation or deprivation of oxygen. In Chiray's experiments glycuronic acid was produced by administering camphor by mouth or the injection of camphorated oil in dogs, rabbits, guinea pigs and man. The reaction reached a maximum at about the third hour. With marked insufficiency of the liver there was no response to the ingestion of 0.5-1.0 gram of camphor.

Camphor administration to dogs by Mandel and Jackson resulted in decreased glycuronic acid production after glucose feeding, meat causing an increase. A proteinogenous origin of glycuronic acid was thus indicated.

Santonin.—The increase in uric acid excretion after santonin is attributed by Abl to intestinal irritation.

Body Temperature.—Many so-called "convulsant poisons," including strychnin, santonin, picrotoxin, camphor, phenol, etc., have been shown by Harnack to produce characteristic changes in the heat regulation. The salient result is a fall in body temperature. Small doses cause increased heat loss and a slightly smaller heat production.

Larger doses cause increased metabolism, through muscular action, (both heat production and loss being thus increased). Paralytic doses diminish the heat production very greatly.

The temperature accordingly varies, but the smallest and the largest doses lower it decidedly. The heat loss is seen especially in small and young animals, larger animals showing some temperature rise with the medium doses.

Curare.—This poison as is well known paralyzes all voluntary motor nerve endings. Asphyxia therefore results by the interference thus produced with the external respiratory mechanism. The salient feature of its action upon the metabolism is the glycosuria, discovered by Claude Bernard. Penzoldt and Fleischer first called attention to the importance of asphyxia as a causative factor. Araki pointed out its relation to the

liver glycogen. MacLeod failed to produce glycosuria either by asphyxia or by curare in Eck-fistula dogs after ligation of the hepatic artery. Since, however, he was unable to prevent curare glycosuria entirely by employing adequate artificial respiration, some other factor besides asphyxia must be involved.

Diminution in the total metabolism was claimed by Röhrig and N. Zuntz and others, who found a decrease of fifty per cent in the respiratory exchange of rabbits. But O. Frank, Voit and Gebhard found no essential difference between normal and curarized dogs when precautions were taken to keep the body temperature from falling. Tangl has recently confirmed this observation.

The nitrogen metabolism has been stated to be reduced by curare, but this effect appears to have been simulated by a simple delay in excretion (Voit).

Body Temperature.—The experiments of Röhrig and Zuntz were the first in which it became clear that curarized mammals become poikilothermic at ordinary room temperatures.

Krogh states that the curve of oxygen absorption as influenced by body temperature is the same in anesthetized cold-blooded animals as in the curarized dog.

Cocain.—*Body Temperature and Heat Production.*—The hyperthermia which cocain induces, while accompanied by greatly increased muscular movements, can best be accounted for by the loss of much fluid from the blood. (Unpublished work of the author.) The temperature rise, according to Mosso, can be prevented by curare or chloral but not by the antipyretics. In dogs Reichert found that ten milligrams of cocain per kilo given subcutaneously caused in one hour a mean maximum increase of 146.9 per cent in heat produced and a mean maximum rise of 1.81° in temperature. He observed that cocain is sufficiently powerful to counteract the profound depressant actions of morphin upon heat production and body temperature. The action is a central one, not occurring in the absence of the cerebral hemispheres and basal ganglia.

In one experiment by Kopciowski in a fasting human subject a small dose of cocain diminished the carbon dioxid output by thirteen per cent.

Nitrogen and Fat Metabolism.—Maestro described a nitrogen retention in rabbits associated with oliguria. Large doses (20 milligrams per kilo), as shown by Underhill and Black, lower both nitrogen and fat utilization in dogs.

Carbohydrate Metabolism.—Cocain glycosuria occurs infrequently. Schaer states that the hyperglycemia, in cats at least, when present is due to excitement. In well-fed dogs and rabbits, but not in the starving condition, Underhill and Black found a marked increase in the lactic acid of the urine. They were inclined to associate this with muscular

activity and to ascribe its origin to more than a single antecedent. The ammonia output appeared to bear little relation to the lactic acid elimination.

Purins.—The chief therapeutic value of the purin bases lies in their diuretic property which quite possibly plays the chief rôle in all of their effects upon the metabolism.

Water Metabolism.—In purin diuresis the water of the urine is increased proportionately more than the solids, which also show an absolute increase. The extent of water excretion depends much upon the supply. Widmer(*a*), for example, has shown that caffein diuresis is abundant in dropsical conditions, but fails altogether with dry feeding. On the other hand, during the diuresis of diabetes mellitus E. Meyer has shown that caffein produces no further effect. The reputed superiority of theobromin and theocin as compared with caffein Sollmann ascribes to the fact that the last mentioned is possessed of more toxic side actions which prohibit its being administered in such large amounts.

Schroeder(*b*) observed that the water content of rabbit's blood is decreased by ten per cent after an effective caffein diuresis. Spiro states that theocin also lessens the absolute amount of water in the blood besides the percentage concentration of sodium chlorid.

The secretory theory of caffein diuresis was advanced by Schroeder. It received strong support from the experiments of Richards and Plant, in which it was shown that when the in vitro perfusion flow is kept constant caffein increases the artificial urine. On the other hand, there is a mass of evidence which relates purin diuresis to an increased circulation through the kidneys. For a full discussion of the mechanism the reader is referred to Cushny's monograph.

Nephritic Conditions.—Pearce, Hill and Eisenbrey and others have shown that the diuresis fails to occur in experimental glomerular nephritis. Christian has found theocin of little diuretic value in nephritis except in cardiorenal cases with edema. Here he finds that it increases the sodium chlorid excretion and works best when given with digitalis or intermittently.

MacNider finds purin and other diuretics ineffective in anurias produced by anesthetics except in those cases of ether anuria where the alkali reserve has not been depleted.

Zondek has recently observed that in cases of high grade hydropic nephritis many diuretics of the xanthin group cause a decreased flow (with greater concentration) of the urine. This phenomenon, which as yet lacks confirmation, is attributed to "fatigue" of the renal vessels.

To produce full caffein diuresis in man H. L. Taylor finds that at least 0.5 gram four times a day is necessary. Theobromin-sodium-salicylate may safely be given in doses twice as large.

In the human experiments of Means, Aub and DuBois (see below)

the percentage of heat lost in the vaporization of water from the lungs and skin was not significantly altered by caffein.

Body Temperature.—Binz appears to have discovered that caffein hyperthermia, which is not usually intense, regularly results when considerable doses are administered to animals and man. Pilcher found that the lowered temperature of moderate, but not of deep narcosis, could be successfully combated with caffein. Karelkin states that the temperature increase is much greater in thyroideetomized than in normal dogs. The diuretic effect, which concentrates the blood, is probably responsible for the rise in temperature, but this should be determined by experiment.

Mandel observed a correlation between purin excretion and temperature-fall in fevers. He produced fever in monkeys by xanthin injections; xanthin, if given with salicylate, failed to raise the temperature.

Total Metabolism.—Edward Smith in 1859 by a very large number of carefully conducted experiments established the fact that caffein increases the carbon dioxid output. The rise obtained was anywhere from fifteen to thirty per cent. Reichert by direct calorimetry in dogs observed greater increases in the heat production. Using more modern methods Edsall and Means, and Higgins and Means found increases varying from three to fourteen per cent.

Means, Aub and DuBois observed in four normal subjects receiving 8.6 milligrams per kilo of caffein alkaloid an increase of from seven to twenty-three per cent in the basal metabolism. In these elaborate investigations the independent methods of direct and indirect calorimetry gave results which agreed within one per cent.

F. G. Benedict and Carpenter(*b*) found that approximately three hundred and twenty-five grams of hot coffee will increase the basal metabolism eight to nine per cent.

Nitrogen Metabolism.—C. Voit concluded from his experiments that caffein did not alter the nitrogen balance, although there was possibly some increase in the urea excretion. Ribaut found the nitrogen excretion in man but little changed, while it was moderately increased in dogs. In three of their subjects Means, Aub and DuBois found an increase in nitrogen elimination varying from six to thirty-seven per cent. This was attributed to the diuresis.

Farr and Welker state that theocin decreases the nitrogen excretion in both health and renal disease.

Creatin and creatinin elimination were found but slightly altered by Salant and Rieger.

Purin Metabolism.—Mendel and Wardell have shown that the addition of strong coffee infusion to a purin-free diet causes a marked increase in the excretion of uric acid. This increase was not obtained from decaffeinated coffee. The increase was found equal to the quantity of uric

acid which would be obtained by the demethylation and subsequent oxidation of from ten to fifteen per cent of the ingested caffein.

Astolfani maintains that caffein increases hippuric acid synthesis.

Carbohydrate Metabolism.—There is commonly a slight glycosuria (discovered by Jacobj) during caffein and theobromin diuresis. It depends on the presence of liver glycogen according to Richter(*b*), occurring only when there is considerable hyperglycemia (Hirsch). It is usually prevented by section of the splanchnic nerves, as shown by Pollak, and by suprarenal excision (A. Mayer). Theobromin glycosuria is said by Mieulicich to be inhibited by ergotoxin.

Mineral Metabolism.—The purins may increase the salt excretion even when no diuresis is produced, e. g., in diabetes (E. Meyer). According to Saccone, on the other hand, theobromin and caffein may diminish the chlorid excretion independently of the diuretic effect. In rabbits Bock found that theocin increased both potassium and sodium output, but not parallel with the diuresis. Sollmann found that the chlorid-retaining mechanism which becomes broken down in rabbits remains unimpaired in dogs and man.

Alkalinity.—Higgins and Means found that caffein diminishes the alveolar carbon dioxid in man.

Growth.—Nice finds that caffein-fed mice exhibit subnormal activity. Caffein increases their fecundity, but the viability of the young is reduced. The growth of the young is only inhibited if they themselves are fed caffein.

Catalase.—Burge states that blood catalase is increased by caffein and theobromin. Blood concentration was apparently not allowed for. (Stehle(*b*)).

Guanidin Bases.—Watanabe(*c*) finds that the metabolic effects induced by guanidin hydrochlorid resemble those of tetania parathyreopriva. For example, besides the tetany there are an excess ammonia excretion, a low content of calcium associated with high phosphates and a hypoglycemia. Calcium lactate injection, however, fails either to restore the blood sugar content or abolish the tetany.

IX. Endocrin Drugs

Epinephrin.—*Total Metabolism.*—Hàri observed a diminution in the total metabolism when epinephrin was injected into curarized dogs, either intravenously or intraperitoneally.

Later investigators, however, find that the characteristic action is to increase the total oxidations in the body; for example, Tompkins, Sturgis, and Wearn have observed that the basal metabolism is increased after epinephrin not only in normal individuals, but in hyperthyroidism and

in soldiers with "irritable heart." The metabolic increase runs parallel to the circulatory changes. Sandiford finds in man that 0.5 c.c. per kilo of 1-1000 epinephrin injected subcutaneously invariably causes an increase in the metabolic rate. She attributes the increase in heat production to an excess of carbohydrate in the circulation with possibly a direct stimulation of the cells as well. (In addition acid metabolites from circulatory stimulation are presumably involved, as is the case with the increase in oxidations produced by tyramin.)

Evans and Ogawa found the total gas exchange of the heart notably augmented.

Catalase.—Burge(*b*) states that the injection of epinephrin stimulates the catalase output of the liver. Stehle believes that Burge's results here and elsewhere are merely an expression of the red blood cell count; "high catalase" would then be equivalent as a rule to blood concentration, "low catalase" to dilution.

Body Temperature.—It has long been known that large doses of epinephrin cause collapse with a fall in body temperature. Freund observed, however, an increased temperature in rabbits on a dry diet with little change in temperature on a green diet. His correlation of epinephrin fever to that produced by sugar or salt has been mentioned.

Hirsch found a decrease of temperature after epinephrin, ascribing it to lowered heat production. Kondo in rabbits found no effect with small doses, but depression of temperature when more epinephrin was given; on the other hand, after thyroid preparations or peptone, and sometimes after atropin, epinephrin raised the temperature.. Intracerebral injections in his hands gave a marked increase in temperature with small or large doses. This effect was somewhat antagonized by antipryin or by thyroidectomy. Barbour and Wing, however, reduced the temperature by intracerebral injections of epinephrin.

Hultgreen and Andersson first showed that adrenalectomy reduced the temperature. Freund and Marchand found that removal of both adrenals results in gradual diminution of body temperature and that the blood sugar at the same time may fall as low as .01 per cent.

Water Metabolism.—While some of the earlier investigators maintained that epinephrin causes diuresis, it is now generally believed to exert, temporarily at least, an opposite effect. Gunning, for instance, finds that intravenously given in all effective doses epinephrin lowers the urine flow both in anesthetized and unanesthetized dogs. The effect is probably associated with renal vasoconstriction.

Lamson and Keith have shown that epinephrin increases the red blood cell count, which phenomenon is associated, in part at least, with diminution of the blood volume. The water passes into the lymphatic system, particularly of the liver. In some species these effects fail to appear.

Carbohydrate Metabolism.—Epinephrin glycosuria has received much

attention since its discovery by Blum. Hyperglycemia was observed by Zuelzer, Vosburgh and Richards and others. Doyon, Morel and Kareff showed that glycogen is simultaneously lost from the liver. Iwanoff demonstrated that epinephrin perfused through surviving livers stimulates sugar formation, thus showing that the point of action is peripheral. The glycosuria is not asphyxial, but nervous stimulation of the adrenals may contribute to asphyxial glycosuria. (MacLeod and Pearce.)

Pollak(*a*) finds that epinephrin glycosuria fails after repeated injections, as the glycogen becomes exhausted. Kuriyama has shown that epinephrin does not interfere with the storage of glycogen by the liver, earlier investigators having neglected the factor of malnutrition in their animals.

Lusk demonstrated that epinephrin does not influence the oxidation of injected glucose; in dogs the respiratory quotient rises to unity either with or without the drug. Furthermore, Fuchs and Roth obtained the following respiratory quotients in human beings with subcutaneous injections of epinephrin alone:

Before: 0.85-0.87; *during effect,* 0.91-0.96; *after,* 0.84-0.86.

Evans and Ogawa from experiments upon isolated mammalian hearts concluded that epinephrin does not alter the power of the tissues to use carbohydrate.

Protein Metabolism.—Lusk has shown that there is no significant change in the protein metabolism after epinephrin. The urea changes noted are apparently due to renal effects. Addis, Barnett, and Shevky observed increases in urea after subcutaneous injections of epinephrin; but large amounts of the drug decreased the urea excretion of dogs. Uric acid and allantoin excretion are stimulated by large doses, according to Falta.

Mineral Metabolism.—Bulcke and Weiss described an inhibition of sodium chlorid excretion under epinephrin. Schittenhelm and Schlecht found that in "war edema" epinephrin (which apparently failed to raise the blood pressure under the conditions) had a tendency to lower the excretion of water and of chlorids.

Growth.—Chambers observed that suprarenal extract increases the rate of division in paramecia.

Epinephrinemia from Drugs.—Stewart and Rogoff(*a*) have recently described an increased output of epinephrin from the adrenal glands under the influence of a variety of drugs. These results must often be taken into account in the interpretation of the action of such substances.

Thyroid Gland Substance.—To combat the effects of thyroid deficiency the administration of thyroid gland substance offers one of the most striking achievements of modern therapeutics. The first patient thus treated has just died at the age of seventy-one after enjoying twenty-eight years under continuous treatment by Murray. The isolation of thyroxin by Kendall has made available a crystalline substance the chemical structure of which is under investigation.

Total Metabolism.—Recent investigation by DuBois, by Means and Aub and others have shown that the basal metabolism is a most important feature of Basedow's disease. It was first emphasized by Magnus-Levy. DuBois showed that heat production is fifty per cent above the normal in severe and seventy-five per cent in very severe cases. This test is proving of value in indicating the proper treatment.

In eight cretins Snell, Ford and Rowntree have found that the basal metabolism varied between —7 and —25. By administering four to five

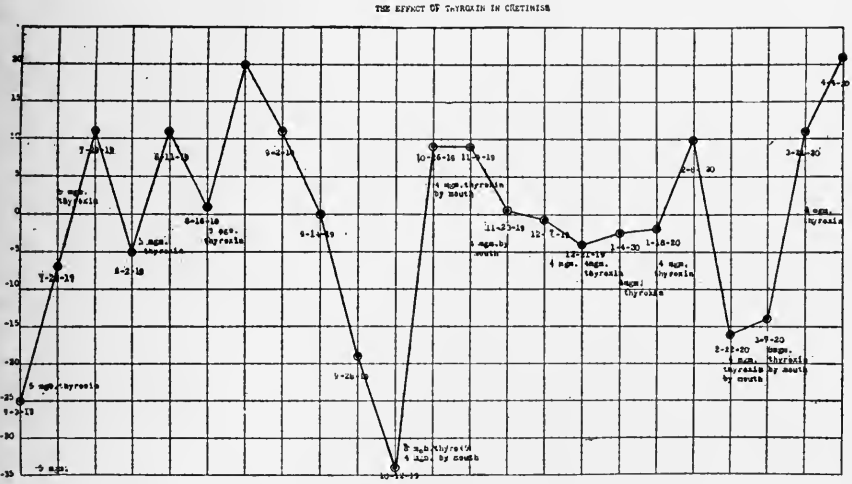

Fig. 5. Effect of thyroxin in cretinism. (A. M. Snell, F. Ford, & L. G. Rowntree, J. Am. M. Assn., 1920, LXXV.)

milligrams of thyroxin every few days these investigators have been able to keep the metabolism close to the normal range. (See Fig. 5.)

Protein Metabolism.—Thyroid administration increases the excretion of nitrogen as shown by Rohde, Stockholm and others. The appetite is usually improved, but there is rapid loss of weight (Leichtenstern). The first effect is on fat, the proteins being drawn upon when the fat is reduced to a certain minimum. On a meat-free diet, according to Krause and Cramer, the nitrogen increase concerns especially the urea, ammonia and creatin, the uric acid and creatinin being very little changed. Kojima finds that thyroidectomized rats excrete less nitrogen and calcium than normally. Curiously thyroid feeding in such animals appears to reduce nitrogen and gaseous metabolism as well as body weight.

Studzinsky and Kaminsky found that thyroid increases the urate excretion in hypothyroidism but not in normal subjects.

Carbohydrate Metabolism.—Thyroidectomized dogs do not utilize sugar as well as normal animals, according to Underhill and Saiki. This

was not found in rats by Cramer and McCall. Watanabe finds the blood sugar and diastase unaltered.

Denis, Aub and Minot have shown that glucose tolerance may be used as a diagnostic test in thyroid disease. The blood sugar is taken as the criterion.

Fat Metabolism.—Thyroid substances must be employed only with great caution if at all to reduce obese conditions not due to thyroid deficiency.

Growth.—Gudernatsch discovered that thyroid feeding retards growth but hastens development in frog larvæ.

Pituitary Substance.—*Total Metabolism.*—No significant effect upon the basal metabolism, according to Snell, Ford and Rowntree, is exerted by the administration of pituitary substance.

Water Metabolism.—While some observers have described fleeting diuretic effects with pituitary extract its most striking influence is antagonistic to the flow of urine. This is seen, for example, in rabbits, which under the influence of the drug give no significant diuretic response to administration of large amounts of water. (Motzfeldt.) Rees finds no alteration of the daily urine output in cats under pituitary treatment. The antidiuretic effect lasts but several hours. Diuresis due to continuous intravenous injection of saline was not affected. Konschegg and Schuster find that one to two c.c. given to normal individuals diminish both the volume and the solids of the urine, the effect lasting sixteen hours.

In diabetes insipidus injections of pituitary reduce materially the volume of urine and the thirst.

Barker and Mosenthal found that subcutaneous daily injections of at least two one c.c. doses of pituitary extract (pars posterior and pars intermedia) were effective in diabetes insipidus over a long period. The urine was diminished in amount, its specific gravity raised; the percentages of sodium chlorid and of nitrogen became increased. Tethelin treatment was not successful nor was the posterior lobe extract of any value by mouth.

Kennaway and Mottram also found subcutaneous injections of pituitary extract effective in diabetes insipidus while orally it was valueless.

Clausen found in a boy of nine and one-half years the usual reduction in fluid excretion by the kidney after pituitary treatment in diabetes insipidus; the hourly chlorid excretion was much reduced. The hourly excretion of urea, creatinin, uric acid and titratable acids was, on the other hand, but slightly affected.

According to Leschke midbrain and not pituitary disturbances are responsible for diabetes inspidus.

The galactagogic effect of pituitary is probably not secretory but due merely to contraction of the smooth muscle of the glands. (Gaines.)

Carbohydrate Metabolism.—Pituitary substance does not alter the blood content in diastase or sugar. (Watanabe.)

Anterior Pituitary Lobe.—Robertson found that feeding the anterior lobe before adolescence retards growth. In adult animals growth however may be renewed. In mice growth retardation is followed by acceleration, especially when tethelin is used.

Partial removal of the anterior lobe of the pituitary leads to obesity and other nutritional derangements. Total metabolism, body temperature and growth become subnormal, as shown by Crowe, Cushing and Homans, and F. G. Benedict and Homans.

In *acromegaly*, which is associated with hyperactivity of the anterior lobe, Bergeim, Stewart and Hawk found no change in the nitrogen or sulphur metabolism, but have described a retention of calcium, magnesium and phosphorus.

Labbe and Langlois abolished glycosuria in a diabetic acromegalic by a four months' course of hypophyseal therapy. The polyuria was not affected.

Other Gland Products—Thymus Gland.—Feeding thymus to amphibian larvæ retards development while hastening growth. (Gudernatsch.) According to Uhlenhuth(*a*) this gland secretes the substance which induces the low calcium metabolism of parathyroid tetany. Thymus injections produce emaciation and malnutrition in guinea-pigs, according to Olkon.

Parathyroid Gland.—The relation to tetany has been referred to in connection with calcium salts. Excision of the gland lowers carbohydrate tolerance, as shown by Underhill and Hilditch. Koch(*b*) found that removal of the parathyroid leads to the appearance of toxic bases (guanidin, histamin, etc.) in the urine.

In parathyreoprival tetany injections of horse parathyroids reduced the creatinin excretion from 1342 to 612 milligrams per day. In rats Kojima found the calcium excretion increased after parathyroidectomy.

Spleen.—Asher and his pupils have recently observed that removal of the spleen augments the respiratory exchange in rats. He regards this organ as antagonistic to the thyroid. While thyroidless rats appear to tolerate low pressure (oxygen-lack) better than normal, the tolerance of spleenless animals is weakened.

Prostate Gland.—Macht showed recently that prostate feeding stimulates both growth and development in amphibian larvæ.

Testis.—Castration of male rats results in diminished oxidations. (Agnoletti, Kojima.) Jean found an increased phosphate excretion.

Pineal Gland.—In animals administration of pineal extracts is said to hasten growth and development. (McCord.)

The Intravenous Injection of Fluids..... *Arlie V. Bock*

Introduction—The Fluids of the Body—The Uses of Intravenous Infusions—Intravenous Infusions to Increase the Volume of Blood and Tissue Fluid—Intravenous Infusions to Increase the Buffer Action of the Blood in Acidosis—Intravenous Infusions to Combat Toxemia—Intravenous Infusions to Assist in Providing for the Calorific Requirements of the Body—Solutions Used for Intravenous Infusions—"Saline" Solutions—Gum Acacia or Gum-saline Solutions—Gelatin Solutions—Sodium Bicarbonate Solutions—Glucose Solutions—Other Solutions—Reactions Due to Infusions—Preparation of Infusion Solutions and Technic of Administration.

The Intravenous Injection of Fluids

ARLIE V. BOCK

BOSTON

Introduction

The rapid adoption of intravenous therapy has resulted from the development of the technic of venous puncture. The simplicity of intravenous injection for the administration of drugs and fluids has secured for this method a wide field of usefulness. In the following pages the use of immune sera and of drugs will not be considered, but attention will be paid rather to the use of injections or infusions of various solutions into the blood stream for the treatment of certain clinical conditions.

The Fluids of the Body

Before entering in detail upon the subject of infusions, the rôle of fluids in the organism will be briefly discussed. It is estimated that the fluid content of the body is equal to from 60 per cent to 70 per cent of the body weight. This fluid consists of the blood, the lymph, and the tissue fluid, all of which may be regarded as mobile fluids, and the fluid within the cells which, in contrast to the rest, is comparatively fixed. The importance of water in the maintenance of life has been emphasized by Starling(*a*), who points out that all of the energies manifested by living cells are derived from substances in solution, and that all metabolic changes in the body relate to changes in and between substances in solution. The organism as a whole strives to maintain a fairly constant quantity of total fluid, as well as to guard carefully the chemical constitution of the fluid in the various systems. This control, although exceedingly complex, since it involves physical and chemical phenomena of an infinite order, and the coöperation of highly organized absorbing and excreting organs, is nevertheless remarkably efficient.

Starling has also discussed the importance of the body fluids in general, from the point of view of the variety of their adjustments to local conditions, by which the cells of the body are enabled to carry out the functions for which they have been differentiated. He has

suggested that the ability of man to withstand changes in his environment, such as extremes of heat and cold, is due to adjustments made by the body fluid to meet the altered conditions. It is this facility to maintain optimum conditions for cellular activity, together with the regulation of the total volume of body fluids that enables all higher forms of life to exist in comfort within the environment.

The cellular fluid has been spoken of as fixed, in comparison with the blood, for example. There is, however, a constant interchange between the cells and the tissue fluid which is of necessity a local interchange. With the details of cellular activity the present discussion is not concerned.

With regard to lymphatic fluid, it need only be said that it represents tissue fluid collected into organized channels, to be returned to the cardiovascular system in order to complete the major part of the circulatory exchange of fluid in the tissues which began with the passage of nutrient fluid from the capillary walls.

The tissues everywhere throughout the body are bathed in fluid that fills the tissue spaces. Since the metabolism of tissue cells is carried on through the activity of this medium the tissue fluid, in a sense, becomes the most important of the body fluids, as Starling suggests. This fluid traverses the system of tissue spaces that form a rather complete circulatory system which, as Meltzer(*b*) has shown, may be in part independent of the cardiovascular system. When the normal quantity of tissue fluid is greatly altered through defect in absorption, or in elimination of fluid, or by direct loss of fluid, there are definite symptoms traceable to such a disturbance. The importance of the tissue fluid which is the last vehicle for the transport of nutrient material to the cell, and the first to receive the waste products of metabolism, cannot be too much emphasized.

Of all the body fluids, the blood occupies the first place in the minds of clinicians, and yet it is only one unit of the various fluid phases within the body. It exerts, however, the controlling influence in the maintenance of function in the normal organism. It is the main highway in the body for distribution and elimination. Of its many characteristics we are here concerned mainly with the question of the volume of the blood. This is roughly one-eighth of the total fluid in the body, and has been found in the normal individual to be a surprisingly constant quantity, subject only to minor variations. Even in disease the variation from the normal quantity is not often great. When the body is confronted with a loss of fluid, such as may occur in severe diarrhea, fluid is withdrawn from the tissue fluid to the blood. This is done in an effort to maintain nutrition of the higher centers at the expense of the tissues in general. Thus, individual cells may begin to suffer from failure of nutrition long before the blood itself shows much evidence of depletion of fluid. This mechanism needs to be appreciated, since conditions in which actual concen-

tration of blood occurs are usually extreme clinical states which may have been avoided by the administration of sufficient fluid.

The intake of fluids is achieved normally by absorption from the intestinal tract. This absorption occurs independent of the body needs, and any excess fluid is readily eliminated by the kidneys. If the rate of fluid intake exceeds the rate of elimination through the kidneys, the tissues become a reservoir temporarily for such excess fluid which is later reabsorbed from the tissue spaces into the blood and passed out through the kidneys. The ingestion of large quantities of water, therefore, has almost no effect in altering the quantity of circulating blood in the normal individual, as shown by Haldane and Priestley. In pathological conditions the same regulation of blood volume tends to occur.

Fluid loss from the body occurs to a certain extent through the lungs and skin. The bulk of fluid, however, is eliminated by the kidneys. The kidneys are responsive to changes in the blood, and their activity in the secretion of urine is the best index as to the state of water balance in the body. Experience has shown that if the intake of food and fluids is sufficient to produce a daily urine output of at least 1,500 c.c. (for an adult), the total volume of body fluids is approximately normal. When the daily urine output falls below 1,500 c.c. it usually does so because the intake of fluids as such, together with the water contained in the food ingested, is not great enough for the needs of the body. Cases of anuria due to nephritis, and cases of cardiac failure of the congestion type, for example, are exceptions to this rule for obvious reasons. The practical importance, therefore, of measuring the amount of urine voided in twenty-four hours in almost all cases of acute illness is that it provides direct evidence as to whether or not the body is being furnished with an adequate supply of fluid.

The Uses of Intravenous Infusions

Intravenous injections are employed usually for four main purposes: (1) to increase the volume of the blood and tissue fluids of the body; (2) to increase the buffer action of the blood in acidosis; (3) to combat toxemia by what is generally regarded as a washing out process; (4) to assist in providing for the calorific requirements of the body.

1. *Intravenous Infusions to Increase the Volume of Blood and Tissue Fluid.*—The following conditions may deplete the store of fluids in the body: (A) fluid loss by (1) hemorrhage, (2) abnormal sweating, (3) severe diarrhea, and (4) polyuria; (B) insufficient fluid intake by (1) starvation, (2) inanition, (3) vomiting, (4) coma, and (5) delirium. The chief symptom manifested as a result of dehydration of tissues in these conditions is thirst, which constitutes nature's indication for treat-

ment. An attempt to restore the fluid loss in all of these conditions may be made by giving fluid by one or another of the following methods: by mouth or rectum, permitting absorption from the alimentary tract; by subcutaneous injections, intraperitoneal injections, or intravenous infusions. The method adopted will depend upon individual indications.

In the case of acute hemorrhage, dilution of the blood rapidly occurs by transfer of tissue fluid to the vascular system, and the original volume of the blood plasma is promptly restored, if the hemorrhage is not too great, and if the supply of tissue fluids is normal. The chief danger in acute hemorrhage is due to the rapidity with which blood is lost, rather than the amount of blood released from the circulation. If hemorrhage occurs so suddenly that compensatory mechanisms such as vasoconstriction and tissue fluid dilution cannot maintain the blood pressure at a safe level, transfusion of blood, or intravenous infusion, may be immediately urgent. Complete collapse of patients after hemorrhage is often the result of the concurrent factor of shock, by which the volume of blood tends to be still further diminished. When shock is present the transfusion of blood, or the infusion of a fluid substitute for blood, may be obligatory. A falling blood pressure is a positive indication for such treatment in order to relieve the anoxemia,[1] particularly of the vital centers. A transfusion of blood, or an infusion under such circumstances, by increasing the volume of fluid in the vascular bed, increases the volume output of the heart per systole, and thus tends to restore the arterial pressure to a normal figure. If a state of shock has existed for several hours the transfusion of blood should always be carried out in preference to other intravenous therapy. In cases of hemorrhage, in addition to transfusion or infusion, an abundant fluid intake by the alimentary tract should be maintained in order to satisfy completely, not only the blood plasma volume, but the supply of tissue fluid as well. The increased efficiency of the circulation, and the good effect upon the rate of blood regeneration as a result of a forced fluid intake in cases of hemorrhage has been recently discussed by Bock and Robertson.

The question of the use of infusion for the treatment of acute hemorrhage and shock presents a problem not common to other conditions for which infusions may be indicated, namely, the necessity for an immediate increase in the total mass of circulating blood. Reduction in blood volume below a certain level results in a fall of blood pressure, accompanied by the attendant difficulties which this failure of the circulation imposes upon the organism. In order to restore the efficiency of the circulation, the volume of the blood must be largely restored as rapidly as possible either by transfusion of blood or by the intravenous infusion of a fluid substitute. In addition to the transfusion of blood, which is the most

[1] A comprehensive discussion by J. S. Haldane of the cause and effect of anoxemia or oxygen want may be found in the British Med. Jour., 1919, 2, pp. 65-71.

effective measure, many solutions have been used to accomplish this end. In the case of a fluid substitute for blood, the solution, according to Bayliss(*c*), should possess the same viscosity as blood, in order to raise the blood pressure to a normal level, and to exert the same osmotic pressure as the colloids of the blood plasma, which will prevent the loss of fluid from the circulation. If a solution possesses these properties it will tend to maintain the blood pressure at a normal level for many hours, because the volume of fluid injected remains in the blood vessels for an indefinite time. In order to insure this result, the solution, furthermore, must be colloidal in nature, since the capillary walls are relatively impervious to colloids. The best solution of this nature yet proposed is one containing gum acacia, to the strength of 6 per cent to 7 per cent in 0.9 per cent saline (gum-saline), as described by Bayliss(*c*). Rous and Wilson, on the other hand, state that a fluid substitute for blood need not have the same viscosity as whole blood. They removed as much as 75 per cent of the hemoglobin of rabbits by bleeding and replaced the volume by rabbit's plasma. No great change was observed in the behavior of these animals. However, the fact remains that no artificial solution of low viscosity used up to the present time has proved to be so useful for the treatment of hemorrhage and shock as the solution recommended by Bayliss.

Of other colloidal solutions, gelatin in 2.5 per cent solution as recommended by Hogan in 1915 has been found useful. More recently, Erlanger and Gasser have proposed the simultaneous use of hypertonic gum-salt solution and hypertonic glucose solution. They have used an 18 per cent solution of glucose and a 25 per cent solution of gum-saline with good results for the treatment of hemorrhage and shock in dogs, and also in a small series of human beings. The beneficial effects thus obtained are explained in part by these authors as due to the internal transfusion effected by the hypertonic solution of glucose, resulting in a still further expansion of the blood volume. This secondary increase of volume is maintained by the hydration of the excessive amount of gum acacia present in the circulation.

The failure of isotonic salt solution to maintain blood pressure after hemorrhage is well known. Physiologists have long ago shown that the introduction of normal saline into the blood stream has only a fleeting effect upon the blood pressure, because this fluid leaves the blood stream for the tissues and urine within a few minutes after it is injected. The reason for this is the low viscosity of the solution as compared with blood, together with the fact that the walls of the capillaries are especially permeable to all crystalloids. Modifications of normal saline, such as Ringer's solution, hypertonic and hypotonic salt solutions, share the same fate as normal saline.

It is to be remembered that all artificial fluids are substitutes for blood, and that in the treatment of hemorrhage, transfusion of blood is

the most efficient therapy in all severe cases. In shock without hemorrhage intravenous injection of a fluid substitute for blood is indicated.

In conditions other than hemorrhage and shock, in which fluid depletion occurs, there is not usually the urgent necessity for an immediate increase of the volume of the blood. Dehydration of the tissues in general, however, is always a serious matter and demands energetic measures to combat the deficit of fluid. Such fluid loss is met with in conditions mentioned on page 789. To increase the store of body fluids in such states it may be necessary to use one or more of the following absorption routes: from the gastro-intestinal tract, which is the one of choice; by subcutaneous injection, or intravenous infusion. If the treatment is necessary because of vomiting, for example, large amounts of normal saline may be absorbed from the subpectoral areas. Injections of this type may be repeated as frequently as absorption occurs. If conditions prevent the use of the alimentary tract, the same object can be achieved with more comfort to the patient by the intravenous injection of fluids such as normal saline or glucose solutions. Intravenous injection of suitable amounts of fluid may be repeated every four hours.

2. *Intravenous Infusions to Increase the Buffer Action of the Blood in Acidosis.*—It is not intended here to discuss the question of acid intoxication in the body. However, the intravenous use of solutions of sodium bicarbonate in combating acidosis requires a brief discussion of the basis for the use of alkali in this condition. Henderson(*b*) has shown the importance of the phosphates and carbonates in maintaining a constant reaction of the blood. These bases exist in balanced solution in the blood, and are able to take up relatively large quantities of acid or alkali without greatly altering its normal alkalinity. This mechanism, together with a similar action of the proteins of the blood, constitutes the buffer action of the blood. For practical purposes the buffer salts may be regarded as bicarbonates. They may be measured in terms of carbon dioxid, with which they combine, by the method of Van Slyke(*b*) or Y. Henderson and Morris. The constancy of the reaction of the blood is maintained chiefly by the elimination of carbon dioxid in the lungs, and of acid radicals by the kidneys. In each cycle of blood the bases thus tend to be conserved in the body. In pathological conditions extreme depletion of the bases may occur in an attempt to maintain the normal reaction of the blood. In these conditions the administration of alkali is advocated in order to renew the lost bases from the blood and tissues, as well as to neutralize non-volatile acids being formed in the body.

Theoretically, the administration of an alkali such as sodium bicarbonate, first suggested by Stadlemann(*a*) in 1883, should be an efficient means of restoring the alkali reserve of the body, and thus become an aid in the treatment of the acidosis associated with diabetes. The earlier, almost universal, use of bicarbonate for the treatment of this condition, how-

ever, has been given up, not only because it does not control the acidosis but also because it produces deleterious effects. Allen, Stillman and Fitz found that high dosage of bicarbonate by mouth seemed necessary in certain cases, but that its intravenous use failed to save any patients in their series of cases. They emphasize the danger of the abuse of sodium bicarbonate in the treatment of diabetes, and in general deprecate its use at all. Joslin has also discussed the harmfulness of sodium bicarbonate and does not use it in the treatment of diabetes.

Beneficial results from infusion of solutions of sodium bicarbonate in cases of acute nephritis complicating cholera, as well as in certain types of nephritis from other causes have been reported by Sellards. The cases of chronic nephritis which he treated required the intravenous injection of as much as 150 grams of bicarbonate to produce an alkaline reaction of the urine, in contrast to a normal tolerance of 5-10 grams by mouth. Howland and Marriott(*c*) also have found sodium bicarbonate infusions useful in the treatment of acidosis incident to diarrheas of infancy and childhood. Its use is advocated by Wright and Fleming for the treatment of gas gangrene in which, in severe cases, there is a great reduction of the alkali reserve. Cannon, Fraser and Hooper used bicarbonate in the treatment of the acidosis accompanying shock, but a later paper by the British Medical Research Committee asserts that the restoration of the circulation by means of transfusion, etc., renders the use of alkali unnecessary in this condition.

Good results from alkali therapy may be expected usually only in the treatment of cases of acute acidosis, the development of which has been so rapid that the chemistry of the body has not had time to compensate for the changed conditions. Examples of this type are seen in methyl alcohol poisoning and acute uremia. In such conditions, in addition to alkali therapy, forced elimination is also essential.

The practice of administering bicarbonate as routine before and after surgical procedures has no justification except in the case of a considerable deficit of alkali. Caldwell and Cleveland determined the change in the plasma carbon dioxid before, during and after surgical operations, and concluded that the diminution in the alkaline reserve below the average normal does not reach the point at which the earliest clinical symptoms are observed to occur, namely, about 35 volumes per cent of carbon dioxid. There is at present no indication for the use of bicarbonate by mouth, or intravenously, unless an alkaline deficit is present sufficiently great to produce symptoms. Solutions of bicarbonate have no more effect in maintaining blood pressure than normal saline, according to Bayliss.

If treatment with sodium bicarbonate is instituted, attention should be paid to the reaction of the urine. When this reaction becomes alkaline, the administration of the alkali should be stopped. While the observance of this rule is a safe one for the majority of cases, Palmer and Van

Slyke have shown that in pathological conditions there is danger of giving too much bicarbonate if the administration is continued until the urine becomes alkaline in reaction. An alkalosis may result in such cases, a condition probably not more desirable than the previously existing state of acidosis. For example, Wilson, Stearns and Thurlow have shown the existence of alkalosis in cases of tetany following parathyroidectomy. Tileston has produced tetany in a case of Weil's disease by the overadministration intravenously of sodium bicarbonate, having established thereby an alkalosis of moderate degree. The onset of tetany in a case of bichlorid poisoning after the administration intravenously of 60 grams of bicarbonate has been reported by Harrop(*a*), and Marriott and Howland (see Howland and Marriott(*b*)) have frequently observed the development of symptoms of tetany in infants during the course of bicarbonate treatment. Palmer and Van Slyke suggest that the administration of sodium bicarbonate should be controlled by determinations of the plasma carbon dioxid. The alkali should not be pushed beyond a level of about 70 volumes per cent, which represents the level of plasma carbon dioxid at which normal urine becomes alkaline following the ingestion of bicarbonate.

3. *Intravenous Infusions to Combat Toxemia.*—The importance of an abundant intake of fluids in the treatment of acute toxemia is beyond question. The fact, however, that the gastro-intestinal tract is the natural route for the absorption of fluid is too often overlooked by the advocates of intravenous therapy. Many intravenous infusions could be dispensed with if a sufficient supply of fluid by mouth and by rectum was available. In other words, the failure to recognize the insufficiency of the fluid supply, as well as the excessive loss of fluid that may occur as a result of sweating in a given case, often results in the clinical state for which intravenous infusions become necessary. It is surprising how rapidly and how much fluid may be absorbed from the alimentary tract. When fluid depletion prevails, normal saline, isotonic glucose solution, or tap water, in amounts of 300 to 400 c.c. may be given by rectum every hour for four or five doses, and may be repeated every three hours thereafter if necessary. It should be recognized that many of the conditions requiring increased fluids are ably met by means of absorption from the alimentary canal, and that in many cases in which intravenous infusions are given, the absorption of fluid from the intestine is a valuable adjunct in treatment.

In the event of failure to maintain a sufficient fluid intake by other routes, intravenous infusions in toxemic states should be frequently given. There is a popular belief that intravenous injections of various solutions are capable of washing out toxins from the blood stream and indirectly from the tissues as well. The procedure has been used to diminish the toxemia of pneumonia, typhoid fever, etc. Enriquez has reported good results from the intravenous use of hypertonic glucose solution in the

treatment of a great variety of such conditions. There is, however, no analytical evidence to show that such therapy succeeds in removing from the body substances responsible for the symptoms. Even if dilution of the toxic substances does occur, which is doubtful, it does not follow that their removal from the body is a necessary sequel. All of the symptoms of toxemia are subject to spontaneous changes which make difficult an attempt to judge the value of any single therapeutic measure. There is no reason to believe that intravenous infusions in toxemic conditions have greater value than an abundance of fluid absorbed from the gastro-intestinal tract. The results obtained in the past by intravenous therapy are probably due to the greater facility with which the functions of the body are carried on in the presence of an adequate supply of body fluid.

4. *Intravenous Infusions to assist in providing for the Calorific Requirements of the Body.*—The use of glucose solutions for intravenous therapy has been fostered because of the availability of glucose in processes of metabolism. Unlike sodium chlorid, glucose when introduced into the tissues may be completely burned, and has, therefore, none of the toxic effects associated with sodium chlorid which cannot be destroyed in the tissues. The fuel value of glucose makes its use for purposes of infusion desirable, particularly in conditions in which nutrition for various reasons is not being maintained. Enriquez, by the use of a 30 per cent solution, has introduced intravenously an amount of glucose equivalent to 3,200 calories within twenty-four hours. Glucose requires simple dehydration to transform it to glycogen, and it is a physiologically efficient food substance.

When an isotonic solution of glucose, 5.52 per cent, is injected intravenously, the sugar leaves the blood stream within a very brief period. If a hypertonic solution is injected there is a temporary increase in the blood volume caused by the withdrawal of fluid from the tissues that persists until balanced osmotic relations are again established between the blood and tissues. Usually this adjustment happens within thirty minutes after the injection, but it may require as long as two hours, as shown by von Brasol, Biedl and Kraus, Starling(a) and others. The excess sugar is usually readily stored in the tissues as Kleiner found. The amount of sugar excreted by the kidneys is variable. Kleiner found in dogs that 60 per cent of the injected sugar was excreted in the urine, but the degree of glycosuria and its duration depend not only upon the state of the kidneys and the rate of blood flow, but upon the amount of sugar and the rate at which it is injected as well. After intravenous injection in man, at a tolerant rate of 300 c.c. of a 30 per cent solution, Enriquez found at most 4-5 grams of glucose in the urine during the first two hours after the injection and none thereafter. Woodyatt, Sansum and Wilder, by means of timed injections, have determined the tolerance in man for sugar as 0.85 gram per kilogram per hour. For a

man of 75 kilograms this corresponds to 63 grams of glucose per hour. No sugar appears in the urine and no diuresis occurs at this, or subtolerant rates, since glucose utilization presumably keeps pace with such rates of injection. However, if the rate of administration is increased as high as 5.4 grams per kilogram per hour, glycosuria with an active diuresis occurs, which soon leads to excessive dehydration of the body unless a large amount of water is supplied.

Essentially the same phenomena were observed in dogs by Fisher and Wishart after the ingestion of glucose, but the time relations necessarily extend over longer periods owing to the longer absorption time. Hiller and Mosenthal, however, found in man that ingestion of 100 grams of glucose did not produce hydremia.

The routine use of glucose solutions, instead of normal saline, is now the custom in certain clinics. There is much to be said in favor of this change. Yet too much emphasis has been placed upon the food value of glucose infusions. An intravenous infusion of 500 c.c. of a 10 per cent solution of glucose has a fuel value of only about 200 calories. If such an infusion is repeated every two hours in twenty-four the total calories amount to 2,400. If solutions of greater concentration of glucose are used, correspondingly more time for each infusion must be consumed in injecting the fluid if diuresis and glycosuria are to be avoided. As a practical measure, therefore, the supply of the total calorific needs of the body by means of intravenous injections of glucose is limited to circumstances of an exceptional nature.

Solutions Used for Intravenous Infusions

The use of normal saline for intravenous infusion has formed the basis for the development of other solutions for purposes not served by saline. The following list comprises those solutions that have been found to have the greatest range of usefulness for intravenous injection: (1) "saline" solutions; (2) gum acacia or gum-saline solutions; (3) gelatin solutions; (4) sodium bicarbonate solutions; and (5) glucose solutions.

1. *"Saline" Solutions.*—A solution of normal saline (0.85 per cent sodium chlorid) was first used for intravenous injection. It was found by Sherrington and Copeman and many others, to leave the circulation within a few minutes after injection. This is due to the rapid diffusion of both water and salt until the differences in potential between blood and tissues are again adjusted. When used intravenously for cases of low blood pressure, sodium chlorid has, therefore, only a transitory effect upon the blood pressure. Fraser and Cowell found that such a solution was of little use in the treatment of hemorrhage and shock for this reason, and their experience led them to conclude that the blood soon becomes

more concentrated than it was before the injection. Nevertheless, normal saline may often be used to tide a patient over a critical emergency period, and its usefulness in building up a tissue fluid reserve is established. The work of Bogert, Underhill and Mendel may be referred to in this connection.

Hypertonic solutions of saline tend to produce hydremia, but diffusion processes quickly reduce the level of salt in the blood to the normal, and the excess of water is likewise returned to the tissues, a small amount being eliminated by the kidneys. There is no indication for the intravenous use of hypotonic salt solution.

Sodium chlorid has been shown by Loeb(*a*), Joseph and Meltzer, and others, to possess toxic properties, and clinical experience has also demonstrated this fact. According to Hort and Penfold, undesirable symptoms include fever, rigors, subnormal temperature, diarrhea, intestinal hemorrhages and Cheyne-Stokes respiration. A. S. and H. G. Grünbaum have reported several deaths due to edema of the lungs following the injection of saline solutions in postoperative cases, in which ether was used as the anesthetic, and in which nephritis was also present. On the other hand, Joseph and Meltzer, in experimental work on dogs, rarely encountered edema of the lungs which could be attributed to sodium chlorid. The relation of salt to the edema associated with nephritis, as suggested by Widal and Javal and others, also indicates that an excess of salt may be a source of injury to the patient. Certain histological changes such as vacuolation of liver cells, alteration of red corpuscles, and degenerative changes in heart muscle and capillary walls have been described as due to salt. To the former idea that salt possesses only osmotic properties must therefore be added that of its chemical activity.

When normal saline is injected attention should be given to the amount of fluid used. This should be approximately 1 per cent of the body weight, if rapidly injected into the circulation, but of course may far exceed this amount if sufficient time is allowed for the infusion period. There is almost no danger from embarrassment of the circulation unless very large amounts of fluid are injected rapidly, or unless an injection is undertaken when a patient is suffering from edema of the lungs. It is to be remembered that the capacity of the vascular system is normally much greater than the volume of the blood. The ability of the vascular bed to contract and expand constitutes a valuable compensatory feature of the circulation, as Meltzer has suggested, and it is usually adequate to prevent embarrassment to heart action from intravenous injection of fluid. However, as noted above, salt infusions immediately after anesthesia, in cases having damaged kidneys, should be avoided, as well as giving excessive amounts of sodium chlorid as shown by a fatal case reported by Brooks.

2. *Gum Acacia or Gum-Saline Solutions.*—The use of gum acacia for infusion purposes is a development of the demand during the war for a fluid substitute for blood in the treatment of hemorrhage and shock. According to Bayliss(*c*), gum acacia is a polymerized anhydrid of arabinose. Erlanger and Gasser state that substances similar to gum acacia are widely distributed in the plant kingdom, and are important factors in the nutrition of herbivorous animals. When ingested by man these substances are readily utilized in processes of metabolism. Erlanger and Gasser state that about one-half of the amount of gum acacia injected intravenously is utilized by the organism in the course of twelve hours, but that some of it remains in the body for over forty-eight. Bayliss obtained the pentose test in the blood twenty-four hours after injection of gum-saline.

Gum acacia may be obtained either in the powder form or in lumps (tears). The lump form is usually purer than the powder. For the purpose of infusion, Bayliss found that a solution of gum between 6 per cent and 7 per cent in strength, in a 0.9 per cent solution of sodium chlorid, has the same viscosity as whole blood, and the same osmotic pressure as the colloids of the plasma. Such a solution therefore possesses properties requisite for use in conditions in which an increase in blood volume and sustained elevation of blood pressure are desirable, because it remains in the circulation long enough for the circulatory mechanism to readjust itself. The results obtained by the extensive use of gum-saline by Drummond and Taylor(*d*), and others, justify the theoretical and experimental considerations put forward by Bayliss(*c*). Certain dangers in connection with the use of this solution will be referred to under the subject of reactions.

The quantity of gum-saline which Bayliss recommended for injection is 750 c.c. A safe rule to follow for this solution, as with others for intravenous use, is to govern the amount given in relation to the body weight. A dose equal to 1 per cent of the body weight, to be repeated, if necessary, will usually meet requirements. If a greater addition to the blood volume is desirable, more than this may be given with safety. Gum-saline may be given to cases in shock without overburdening the heart. Its use should be limited to conditions of low blood pressure as a result of hemorrhage and shock. It is not a substitute for red corpuscles and, therefore, can be of no use in treatment for an exsanguinating hemorrhage, for which transfusion of blood alone is indicated.

The use of the combination of hypertonic solutions of gum acacia and glucose, as recommended by Erlanger and Gasser, has not yet been extensively used clinically. When slowly injected, the great viscosity of 25 per cent gum-saline which they used, apparently does not contra-indicate its use.

3. *Gelatin Solutions.*—A solution of gelatin, 2.5 per cent, in normal saline, as recommended by Hogan on account of its colloidal properties,

may be used for the same indications as gum acacia. Hogan demonstrated by blood pressure readings and rate of urinary secretion that this solution remained in the circulation for a considerable period of time. It does not, however, possess the same viscosity as blood. Furthermore, unless special care is taken, heat destroys the colloidal properties of gelatin, upon which its usefulness in this connection depends. Sterilization of the solution also is difficult, owing to the frequent presence of spores of tetanus bacilli. In spite of these disadvantages, gelatin solutions may be of great use if they are made with the precautions suggested by Hogan.

4. *Sodium Bicarbonate Solutions.*—Sodium bicarbonate solutions in strengths varying from 2 per cent to 6 per cent are customarily made up in normal saline. When such a solution is boiled in the process of sterilization, much of the bicarbonate is converted into carbonate. The carbonate is caustic, and is capable of producing extensive sloughing of subcutaneous tissues. It may, however, be injected safely into the blood stream. Carbonates, as such, should not be used as a rule, even for intravenous injection, because of the possibility of infiltration about the vein with consequent tissue destruction. After boiling a solution of sodium bicarbonate, carbon dioxid should be bubbled through the solution to reconvert the carbonate to bicarbonate. Contrary to statements in the literature (Stadlemann(*a*)), not only is the alkalinity of a bicarbonate solution altered by boiling, but also the caustic properties of carbonate in such solutions cannot be neglected. Joslin is authority for the statement that sterilization of bicarbonate is probably not necessary. If not sterilized, it should be handled with sterile utensils and dissolved in sterile normal saline. Solutions of bicarbonate or carbonate should not be injected subcutaneously.

Some of the effects following the injection of sodium bicarbonate are easily measured. The carbon dioxid tension of the alveolar air is increased, the carbon dioxid content of the blood rises, and urine becomes alkaline usually when the tolerance is reached, and in some cases of nephritis, as Sellards has shown, diuresis may be pronounced. Allen, Stillman and Fitz suggest that great care is necessary when sodium bicarbonate is given intravenously, not to force a blood having low alkalinity suddenly to one having a normal or above normal alkalinity. A favorable progress is indicated if the level of bicarbonate tends gradually upward.

5. *Glucose Solutions.*—Glucose is a monosaccharid which shares with fructose the characteristic of being more readily assimilated than any other sugar. It is highly soluble in water, is non-toxic, and may safely be given in concentrations up to 30 per cent to 35 per cent. The isotonic solution is one of 5.52 per cent . When injected into the circulation in isotonic or hypertonic solutions, the excess of sugar is rapidly eliminated

from the blood, a process shown by many observers to be independent of the kidneys and other abdominal organs, and Kleiner has shown that it may to a certain extent be independent of vital function. However, Bogert, Mendel and Underhill, and Boycott and Douglas have found that in animals suffering from acute experimental nephritis, the injected sugar remains for a longer time in the blood, than when the kidneys are normal. This point may be of great clinical importance when such infusions are contemplated for cases of nephritis in man, since it may be associated with the onset of diuresis reported by several observers in cases of anuria.

6. *Other Solutions.*—Certain other substances less widely used for infusion purposes may be mentioned. Intravenous infusions containing calcium and barium have been used for the alleged constricting action of these substances upon arterioles. Bayliss(*c*) has shown that this action lasts but a few minutes and is, therefore, of no great importance. The use of calcium for the treatment of tetany has been suggested by McCallum and Voegtlin, Wilson, Stearns and Thurlow, and others. It is also useful to restore to normal the delayed coagulation time of the blood in cases of obstructive jaundice, as shown by Lee and Vincent. Likewise, the intravenous use of magnesium sulphate for the treatment of tetanus, and for purposes of anesthesia, has been described by Meltzer(*c*) and Auer and Meltzer.

Reactions Due to Infusions

As in the case of blood transfusion, the intravenous injection of solutions is attended with a certain incidence of reactions. In the preceding discussion many of these have already been mentioned. The more common reactions are characterized by symptoms similar to those associated with protein intoxication. The most important cause of these reactions seems related to the water used for the solutions. Chills and fever, resulting from intravenous injections, are for the most part theoretically due to reaction to foreign protein contained in the water. In certain instances, reactions after infusion may be accounted for by the fact that the solution injected was in effect a vaccine and the resulting chill and fever a manifestation of a non-specific immune reaction. In the routine use of infusions experience has shown that chills and fever result in a small percentage of all cases regardless of the type of solutions used. It is well known, however, that in man the rapid ingestion of very large amounts of water may produce the same type of reaction, from which the disturbance may be seen to be a very fundamental one involving the water balance of the body. Hort and Penfold, after carefully investigating the matter, found that water distilled in a glass retort and at

once injected did not produce fever, but tended to cause a fall in temperature. Samples of the same water, collected and sterilized with all the usual precautions and allowed to stand, produced fever upon injection. The cause of such a reaction is unexplained. These authors recommend that water for intravenous use should be recently distilled and sterilized before injection, as the only reliable method of avoiding fever. All water used for infusion purposes should be distilled from water containing as little organic material as possible, and sterilized at once after distillation. It should then be preferably stored on ice if not immediately used.

The bad results that have been reported following the use of gum-saline can generally be explained by investigation of the individual cases. They have been found to be due to the use of impure gum acacia, to improper storage of gum-saline after it has been made up for use, and, as DeKruif showed, to gross infection of the solution. Gum acacia is protein-free and has been demonstrated by Bayliss and DeKruif to be free from anaphylactic phenomena. Before use in man, the toxicity of the stock gum acacia should be tested in cats or guinea pigs. When all precautions have been observed in the preparation of gum-saline, chills have occurred in 5 per cent to 10 per cent of cases after its injection into the circulation. The failure to test the toxicity of the stock supply of gum, and to observe the other usual precautions, has led to some fatalities from its use.

In the case of sodium bicarbonate injections, reactions may consist of convulsions or complete collapse, according to Joslin. The production of tetany after bicarbonate injections has already been discussed. Harrop(*a*) has called attention to the danger of the intravenous use of bicarbonate when the excretory function of the kidneys is impaired, and especially when oliguria or anuria is present.

Chills and fever occurring after intravenous injection of normal saline are usually the result of carelessness in preparation of the solution. The practice of employing as "normal saline" a solution of boiled water plus an indefinite quantity of salt is not to be advised.

Preparation of Infusion Solutions and Technic of Administration

If the general principles concerning the character of water, purity of substances employed, etc., already discussed are followed, no special points remain to be mentioned in the preparation of solutions for intravenous use. The exception to the general rule concerns the preparation of gum-saline, which, owing to difficulties of filtration of the gum, requires special technic. A full description of the method of preparation of gum-saline is given by Telfer.

Solutions for intravenous use should always be made, not only with care as to the character of water used, but also as to the nature and concentration of substances in the solutions. Also, great care must be taken in filtration to remove extraneous or undissolved particles, and in sterilization. The storage of all solutions on ice in the interim before using them is important. Before injection any solution should be warmed to body temperature. In the case of fluids having no greater viscosity than blood, the rate of injection is not significant unless excessive amounts of fluid are given. When amounts of fluid exceeding 1 per cent of body weight, or when solutions of high viscosity are injected, caution as to the rate of injection is necessary. Special care is always advisable when intravenous infusions are given to cases of nephritis.

The methods for administration of intravenous fluid are numerous. The simplest of these depends upon gravity to force fluid into the vein. The syringe method, with a three-way stopcock, so widely used for the administration of salvarsan, is one of the most satisfactory and efficient methods. The apparatus designed by Robertson for the transfusion of citrated blood is also adapted for use with other solutions than blood. In order to introduce known amounts of sugar at a tolerant rate, the method of timed intravenous injections by means of a pump, as devised by Woodyatt, Sansum and Wilder, and later improved by Woodyatt(*b*) is to be recommended.

Artificial Methods of Feeding

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There are times when the need for some method of nourishing the body by other than the normal route is imperative, and has led investigators to determine, if possible, some way that shall be reliable, easy, and capable of supplying at least approximately the needs of the living organism. That it is not reasonable to expect that an individual could be permanently nourished in any artificial way (with the exception of gavage and direct feeding in gastrostomy) goes without saying, but there are some occasions in which an adequate method is indicated—as every clinician can testify. So far, the results of experimentation have been only partially successful, and while it has been found possible to supply practically about one-third the caloric needs of the body, principally in the form of carbohydrate, the problem of furnishing the necessary protein seems still far off.

It has long been known that a man can live many days on his own protein and fat, provided he is given water, and there are numerous instances of professional starvers who have gone forty to fifty days without food, and have come back promptly to normal when they were again fed. In this way we have gained considerable knowledge of the metabolism of starvation over extended periods, a subject which forms an interesting chapter in biological chemistry. The results of fasting experiments in man and animals, Sherman(*a*) says, "show that in fasting the total metabolism continues at a fairly constant rate in spite of the fact that the energy is obtained entirely at the expense of the body material." In long fasts there has been found a somewhat greater decrease in heat production, and Sherman says other factors than the simple sparing of the direct effect of food come into play. Then, too, each type of food exerts a more or less specific influence on energy metabolism, less sugar being required to prevent loss of body substance than fat or protein—an observation of practical importance in devising artificial methods of feeding.

In many of the artificial feeding procedures the metabolism of the body, as shown by the nitrogen balance, body weight and findings of the respiratory chamber, differ little from that found in actual starvation; and although the patients seem to be deriving constructive benefit from

one or another method, accurate data of scientific investigation shows the bettered condition is for the most part only apparent.

The forms of artificial feeding to be discussed are:

1. Gavage.
2. Duodenal feeding.
3. Rectal feeding.
4. Subcutaneous feeding.
5. Intravenous feeding.

Gavage.—By gavage is meant the introduction of food either through the nose or mouth by means of a flexible rubber tube. This is an exceedingly valuable procedure under certain conditions and gives most satisfactory results because the food reaches the gastrointestinal canal through the normal route.

Indications.—The chief indications for the use of this method of feeding are: First, in unconscious patients, particularly in those who have lost the swallowing reflex; second, in the insane who refuse nourishment; third, in conditions of ulceration of mouth or pharynx with painful deglutition; fourth, in babies, at times, who have had cleft palate operations; fifth, in anorexia nervosa where it is necessary to feed in spite of absolute anorexia; sixth, in "hunger strikes," in prisons; seventh, in paralysis of deglutition.

Metabolism.—The metabolism in gavage is precisely that of normal feeding, except that the preliminary mouth digestion is lacking. On this account, foods used in gavage should be either in a liquid form or so finely communicated that they will run through the tube in a liquid medium. The food requirements should be calculated for each patient.

As the psychic stimulus to digestion, so far as taste goes, is not a factor in gavage, it is only necessary to combine the food elements in sufficient amounts and proper proportions to satisfy the nutritional requirements of each case, calculating the caloric value of the foods used on the basis of the patient's activities, according to the well known rules. Thus an insane, hyperactive patient will take many more calories per kg. than one lying unconscious in bed, therefore it is unreasonable to try to supply food formulæ ready made.

Foods Used in Gavage.—The most convenient foods used in gavage are milk, cream, sugars, butter, oils, meat powders, eggs, cereals, cooked starch, etc.

Method of Performing Gavage.—The patient should be placed in as comfortable a position as possible. If in bed, with the head slightly raised; if out of bed best in the upright position; if insane or resisting, tied in bed or to a chair. The tube should be lubricated best with some non-greasy emollient and slipped down the throat at least well beyond

the epiglottis—although not necessarily into the stomach. An ordinary stomach tube may be used or any convenient sized catheter to which is attached a glass funnel. If the tube is passed through the nose, a small sized catheter must be used and the end passed to a point well beyond the epiglottis. Before pouring food into the funnel, one should listen to be sure that the patient is not breathing through the tube, showing it to be in the trachea—a not unusual occurrence, particularly in unconscious patients.

The number of feedings given during the day will depend on circumstances; but three or four feedings in the twenty-four hours should be enough, too frequent passage of the tube being irritating to the mucous membrane. At times it is necessary to insert a mouth gag before passing the tube, and in restless patients who bite the tube it is well to use a spool gag with a good flange, passing the tube through the hole.

Duodenal Feeding.—This method of feeding was devised by Einhorn some years ago, and has found a field of usefulness in certain cases. It has been recommended especially for use in peptic ulcer, chronic gastric dilatation to prevent weight on the gastric walls, allowing them gradually to recover their tonus and contract, provided, of course, the dilatation is not secondary to pyloric obstruction; in cases of difficult nutrition on account of absolute anorexia, nervous vomiting, or asthenia—also in severe hepatic disease when it is supposed to reduce the congestion of that organ—although this is a questionable result; in carcinoma of the stomach where the ingestion of food is painful; in some forms of chronic indigestion.

The metabolism of duodenal feeding is, of course, essentially normal, and follows the same lines as in gavage.

Method of Introducing the Duodenal Tube.—The bulb of the tube is placed in the patient's mouth and a swallow or two of water is given to help in its deglutition—care being taken not to have it swallowed too rapidly as it might curl up in the pharynx. When the tube is in the stomach the patient is placed on the right side, and the tube fed in its entire length, gradually working its way into the duodenum by gravity. The length of time necessary for it to reach the duodenum depends on several factors, on the degree of gastric acidity, the motor power of the stomach muscle and pylorospasm; entering the duodenum most rapidly in hypoacidity when this is associated with good muscle tone and no pyloric contraction either functional or organic. In favorable circumstances, it may enter the duodenum in ten to twenty minutes—possibly two or three hours for normal persons—up to twelve or thirty-six hours in less favorable cases. When the end of the tube has passed the pylorus it is difficult to obtain any fluid and what few drops can be aspirated with a syringe are alkaline and usually contain bile. If the tube is still in the stomach the fluid will probably be acid. If there is an achylia present (and this

acid test of no use) a little milk can be given by mouth or some colored fluid and aspirating at once; if the tube has gone beyond the pylorus no colored fluid or milk will be obtained. The tube's location can also be determined, if necessary, by fluoroscopy after filling it with a solution of barium. The length of time that the tube is left in situ depends on the condition for which it is used, but it can remain for from twelve to fifteen days without detriment, keeping the mouth clean by washes and brush.

Duodenal Feedings.—The feedings recommended by Einhorn consist of milk 210-240 c.c. (7 to 8 ounces), one egg, a tablespoonful of lactose (15 gm. ½ ounce). If the bowels are made too loose, reduce the lactose, and when it is necessary to increase weight, 4 to 8 gm. (1 to 2 drams) of butter may be added to each of the eight feedings given at two-hour intervals. For those patients who cannot take milk, cereal gruels may be substituted, made thin and smooth enough to pass through the tube readily. It will then be necessary to give the protein of the diet in the form of meat powders—egg albumin—or some one of the artificially prepared protein foods, e. g., plasmon, 70 per cent protein; nutrose, 90 per cent protein; beef meal, 77 per cent protein; peptones, e. g., panopeptones, Witte's peptones, Armour's or Cranick's, all of which vary from 1.5 to 10 per cent nitrogen. These latter peptones may easily upset the digestion, causing diarrhea, and are therefore suitable only for short periods. Aleuronat, a vegetable protein, contains 80 to 90 per cent protein, 7 per cent carbohydrate. All these preparations are good as well for reënforcing the milk formulæ.

The food should be given at about 100° F., slowly either by the drop method or by a syringe directly into the tube, or by using a three-way stopcock drawing the food up from a glass. If the food is given rapidly, it distends the duodenum and causes pain. After each feeding saline is run through the tube to cleanse it, followed by air. This is very essential or the tube shortly becomes blocked and has to be removed for further cleaning. Einhorn(*b*) reports 95 per cent of ulcer cases healed at once, and 90 per cent after two years in 132 cases, and other favorable results.

Buckstein(*c*) reports experiences with this method of feeding, using an average mixture of peptonized milk 150 c.c. (5 oz.), glucose 70 gm. (2⅓ oz.), 2 eggs, butter 40 gm. (1⅓ oz.).

Duodenal Feeding—Routine Einhorn Feeding

7:30 a. m.	Oatmeal gruel	180 c.c. (6 oz.)
	One egg	
	Butter	15 gm. (½ oz.)
	Lactose	15 gm. (½ oz.)

9:30 a. m.	Pea soup	180 c.c. (6 oz.)
	One egg	
	Butter	15 gm. (½ oz.)
	Lactose	15 gm. (½ oz.)
11:30 a. m.	Same as at 9:30 a. m.	
1:30 p. m.	Bouillon	180 c.c. (6 oz.)
	One egg	
3:30 p. m.	Oatmeal gruel	180 c.c. (6 oz.)
	Butter	15 gm. (½ oz.)
	One egg	
	Lactose	15 gm. (½ oz.)
5:30 p. m.	Same as at 9:30 a. m.	
9:30 p. m.	Bouillon	180 c.c. (6 oz.)
	One egg	

Total amount:		Calories.
Oatmeal gruel	360 c.c. (12 oz.)	1,476
Eggs	8	800
Pea soup	720 c.c. (24 oz.)	384
Lactose	90 gm. (3 oz.)	369
Bouillon	360 c.c. (12 oz.)	39
Butter	90 gm. (3 oz.)	715
		3,483

This diet list may, of course, be modified downward where fewer calories are needed.

Rectal Feeding.—Rectal feeding has been employed since earliest times in one form or another, and, later, von Leube and Riegel kept patients alive for considerable periods by this method, in one case almost a year, and it was thought it was possible to do this regularly when indicated. Modern scientific experimentation, however, has shown that at best it is a form of partial feeding only, and results in subnutrition. This form of artificial feeding is, nevertheless, the most efficient that we possess so far, and has a field of usefulness in tiding patients over periods when mouth feeding is impossible or inadvisable. The length of time it should be employed and is of practical use is from one to eight weeks, or less; the success of the longer periods is probably due to causes to be dealt with later.

Indications.—The indications for rectal feeding may be summed up as follows: 1. In temporary obstruction from any cause. 2. Inability to swallow, as in stricture of the esophagus. 3. Gastric diseases, e. g.,

ulcer, cancer, pyloric stenosis, protracted vomiting, etc. 4. Increasing emaciation.

Physiology of Rectal Feeding.—The large bowel is ordinarily thought of as a reservoir where the liquid of the chyle, including the salts, is absorbed, where the bacteria continue to break down cellulose, and the feces are compacted. Little if any enzyme action on the foods is carried out except in the ascending colon, where the small intestine digestion is continued for a short time, the large bowel secreting no digestive juices. The substances absorbed are those which travel easiest by osmosis and in the case of rectal enemata reverse peristalsis carries any food solution the whole length of the bowel and into the small intestine if the ileocecal valve is incompetent. It is more than probable in the cases of rectal feeding that have been kept alive for months the success of the procedure has depended on this factor to a large extent, the small intestine being responsible for the greater part of the absorption.

In 1902 Cannon showed by bismuth enemata with food that if small in amount they were carried only to the cecum, but if large and thick, were carried into the small intestine, segmentation taking place following antiperistalsis, particularly if considerable pressure was used in their introduction.

Metabolism of Rectal Alimentation.—As rectal feeding has been subjected to more accurate laboratory methods, the clinical observations indicating almost complete nutrition by this method, have of necessity been modified, and at best it has been found that only about 30 per cent of the total caloric needs of the body can be supplied, save in exceptional cases.

Of the different food elements introduced by enema it is necessary to speak more in detail concerning the fate of protein, carbohydrate, fat, alcohol, salts and water.

Protein.—Almost every conceivable form of protein has been used at one time or another in rectal feeding, and Bauer and Voit(*d*) in 1869 proved by the increase in urinary nitrogen that protein, when properly prepared, was absorbed to some extent.

Edsall and Miller(*e*) found in two patients 3.04 gm. N (19 gm. P) and 3.8 gm. N (23.8 P) absorbed; Boyd in six patients receiving an average of 44.6 gm. protein (7.16 N) there was absorbed 8.87 gm. protein (1.42 N) i. e., 20 per cent of the intake, and the nitrogen balance was in every instance a negative one. Adler, using peptonized milk per rectum, gave 3.9 gm. N, 2 gm. being found in the feces, proving that approximately one-third of the protein was absorbed.

Short and Bywaters(*f*) analyzed reports of cases fed by rectal enema together with weight charts and urinary findings and concluded that: 1. The daily output of urinary nitrogen from patients given enemata of peptonized milk and eggs (peptonized twenty to thirty minutes) showed that almost no nitrogen was absorbed, and the total nitrogen in the urine

was little, if any, higher than that seen in the urine of fasting men or of patients who received only saline by rectum. 2. Modern physiological opinion holds that proteins are absorbed principally as amino-acids, and the failure of the rectum to absorb ordinary nutrient enemata is largely due to the fact that peptones are usually given instead of amino-acids. 3. Chemically prepared amino-acids or milk pancreatized for twenty-four hours, so that the amino-acids are separated, allows a much better absorption of nitrogen as shown by the high nitrogen output in the urine. 4. The low output of ammonia nitrogen shows that the high total nitrogen was not due to the absorption of putrefactive bodies when the amino-acids were used.

Bauer showed that peptones, meat juices and alkali albuminates were absorbed by rectum but only when salt was added, also that propeptones, milk, casein, globulins and egg albumin salted or mixed with pepsin were absorbed.

From the foregoing, it is seen that some confusion still exists as to just how well the various forms of protein are absorbed, but in general it may be said that "the nearer the protein molecule approaches its ultimate fate in normal digestion, i. e., as amino-acids, the better is its absorption." So we find peptone better absorbed than albumin, amino-acids than peptone, the best rate of absorption being seen when salt is added to the enema. Amino-acids may be most conveniently produced by the pancreatization of milk for 24 hours, in which condition a fair amount is absorbed but not enough to prevent a constant negative nitrogen balance. There are also amino-acids produced chemically from beef, but they are not so well borne, causing rectal irritation.

Fats.—The rôle of fats in rectal feeding is a very minor one, and authorities differ again as to this. Friedenwald and Rürah believe that fat in fine emulsion, as in egg yoke, is fairly well absorbed. Short and Bywaters conclude that very little, if any, fat is absorbed, which agrees with Brown's(*g*) observation that fats given by mouth increase fats in the urine, while if given by rectum they do not. There is no objection to using a finely emulsified fat in the nutrient enema, but there is little object in doing so, as dextrose is well absorbed and takes the place of fats in sparing protein.

Carbohydrates.—These, so to speak, form the sheet anchor in rectal feeding and experimental evidence is definite that they are absorbed fairly readily when offered in proper form and concentration. This has been proven, as in giving dextrose the respiratory quotient was raised and acidosis diminished. Even raw starch has been used and not found in the feces, but dextrinized or malted starch is less irritating than the sugars, according to some authorities, and may be used in their stead. Lactose is poorly absorbed, as shown by the rapid rise of ammonia nitrogen in the urine when this was substituted for dextrose, although it is of some

use in milk enemata by its action in reducing fermentation. The monosaccharids are all well absorbed by the colon in considerable quantities, and of them dextrose is the best for general use. Boyd and Robertson found that 9/10 of a 10 to 20 per cent solution of dextrose was absorbed up to 40-50 gm., but decided that a total of 30 gm. was less apt to irritate the colon. Goodall found with a 10 per cent solution 157 to 163 gm. was absorbed and with a 15 per cent solution a total of 144 to 193 gm., not more than 0.5 to 1 per cent being lost by bacterial action. Boyd gave patients an average of 55 gm. dextrose with an average absorption of 53 gm. Gompertz, using a 3 per cent solution gave 60 gm. dextrose and found 52 gm. absorbed in 24 hours, 8 gm. being recovered from the stools; using a 10 per cent solution 200 gm. were given, 163 gm. absorbed; of a 15 per cent solution, 300 gm. were given, 144 gm. absorbed; and alimentary glycosuria did not occur.

For the most part, therefore, it has been found that solutions of dextrose up to 5 per cent were best tolerated and can be used over considerable periods without irritation. If fermentation is a factor it can be controlled by adding 1 part of thymol in 4,000 parts of the solution.

Salts and Water.—It has been abundantly proven that these substances are rapidly absorbed by the rectum and really largely account for the success of rectal feeding. Gompertz(*h*) did experiments with both potassium iodid and sodium chlorid and found both well absorbed. Apparent gains in weight are no doubt due in some instances, as Coleman points out, to water retention.

Formulæ for Rectal Feeding

Among the most easily prepared and satisfactory foods for rectal feeding is milk, preferably skimmed, and pancreatized from 8 to 24 hours, after which enough dextrose is added to make a 5 to 10 per cent solution and salt 5 gm. to the liter. The milk should be scalded after peptonization to sterilize it, and then kept on ice. Of this solution, 6-8 ounces (180-240 c.c.) may be given by rectum every four to six or eight hours, depending on the ability of the patient to take it. This may also be given advantageously by the Murphy drip, thirty-five drops to the minute, three pints or more being given this way in twenty-four hours.

The following combination of dextrose, alcohol and pancreatized milk represents a fair sample formula, although in some patients the alcohol has to be omitted and the lower percentage of dextrose used.

Dextrose	20 to 50 gm.— 80 to	205 calories
Alcohol	20 to 50 gm.—140 to	350 "
Pancreatized milk	1,000 c.c. —650 —	650 "
Salt	5 to 9 gm.	
	870 to	1,205 "

This may be given in a 250 c.c. dose every four to six hours, and if well tolerated aids materially in helping the patient to tide over an emergency. By omitting the milk, the solution is useful in: 1. Simple exhaustion. 2. In septic conditions. 3. As an antidote to chloroform; in phosphorus poisoning; or anything that causes fatty degeneration of the liver, e. g., toxemia of pregnancy. 4. In diabetic acidosis and acetonemia. 5. After abdominal operations, especially in undernourished or desiccated individuals.

Instead of the pancreatized milk, one may use white of egg, plasmon, casein, somatose or aminoids, etc., but they offer no particular advantage over milk and are sometimes irritating to the rectum.

Fitch(*i*) recommends:

Eggs, two whole	100 gm.	160 calories
Dextrose, 1½ teaspoons	6 gm.	30 "
Pancreatized milk, 10 oz....	300 c.c.	210 "
Salt, ½ teaspoon.....	2 gm.	0 "
		400 calories

Cornwall(*j*) uses two formulæ: No. 1 contains protein 20 gm. in amino acids, glucose 90 gm., vitamins, salt and water 1,500 c.c., and 700 calories, given as follows: 6 a. m., glucose 30 gm., strained juice of half an orange, soda bicarbonate 2 gm., salt 2 gm., water q. s. ad 300 c.c.; 8 a. m., 150 c.c. skimmed milk thoroughly pancreatized; 12, same as at 8 a. m.; 4 p. m., same as at 6 a. m.; 6 p. m., same as at 8 a. m.; 10 p. m., same as at 6 a. m.; midnight, same as at 8 a. m.

Every second day, at 4 a. m., a colon irrigation is given with saline 0.9 per cent solution, and the glucose enema at 6 a. m. omitted. The percentage of glucose may be reduced or increased according to reaction. A culture of acidophilic bacteria may be added.

Formula No. 2 supplies 700 calories, salts, vitamins and water 1,800 c.c., but no protein, as follows: 6 a. m., glucose 30 gm., strained juice of half an orange, soda bicarbonate and salt of each 2 gm., water 300 c.c. Repeat this at 10 a. m., 2, 6 and 10 p. m., and 2 a. m.

Precautions and Technic in Rectal Feeding

1. *The rectum must be kept clean by a saline irrigation or enema, once a day.*

2. *All food should be sterilized before injecting.*

3. *If the rectum becomes irritated, give a rest of 6 to 8 hours, or use only saline solution for a time.*

4. *Enemata should be given with the patient on the left side, or with the foot of the bed raised on shockblocks, which are left in place for an hour after the injection.*

5. *In certain cases of excessive peristalsis, it is necessary to use 5 to 10 drops of deodorized tincture of opium in the enemata.*

6. *Injections should be given slowly, the rectal tube lubricated and passed not more than 6 to 8 inches, and the reservoir containing the solution should not be more than 18 inches or two feet above the level of the patient's back.*

7. *All fluids should be as nearly blood temperature as possible on entering the rectum. This can be facilitated by placing an electric light bulb in the reservoir and placing a hot water bag over the feed tube just before it enters the rectum.*

If the Murphy drip method is used, Kemp has devised a special heat retaining bottle to use and has worked out the following table for determining the temperature:

Table of Temperature of Fluid in Bottle	Length of Tube	Number of Drops per Minute	Temp. in Rectum
190° F.	30 inches	60	115° F.
160° F.	" "	20 or less	100° F.
150° F.	" "	40-50	100° F.
140° F.	" "	150-200	105°-110° F.

Summary of Results of Rectal Feeding.—1. Only about 25 to 35 per cent of nourishment required to maintain nitrogenous equilibrium and weight is absorbed per rectum.

2. Metabolism experiments show that even under the best of conditions this method, although the best we have, results in subnutrition, and is really semi-starvation.

3. As a practical method, it should not be relied upon to bring up a patient's condition as, e. g., for an operation except where there has been actual starvation as in a marked esophageal or pyloric stenosis. It is a false prop.

4. It is useful in tiding over short periods when from one reason or another it is necessary to give the patient water, salts, and some nourishment in the form of protein and carbohydrates.

5. Its usefulness is, therefore, limited, more so than many people suppose.

Subcutaneous Feeding.—There are occasions when this form of feeding would be of great value even for a few days if it could be done comfortably and efficiently, but as yet it has not been possible to accomplish this with any degree of satisfaction. Although considerable experimentation has been done towards this end, at present the rectal method is much

more satisfactory and useful and the future will have to determine the possibilities of subcutaneous feeding, although a certain amount can be done in this way now. Any substance used must be capable of direct assimilation, non-irritating and easy of sterilization.

Protein.—Protein has been used in many different forms, as egg albumin, peptone, alkali albuminate and propeptones, but it was found that all these forms of protein lead to severe local reactions—abscess formation and breaking down of the tissues. Experimentally(*k*), it was found possible in dogs by small and repeated injections of skimmed milk peptonized one and a half hours, to supply a certain amount of protein, the nitrogenous balance showing a loss of only 0.3 to 0.5 gm. per day. These injections were toxic and particularly so unless the dose was begun low and very gradually increased, so that this form of protein is not practical and should not be used. Ascitic fluid and blood serum have also been used with better result and a certain amount of protein can be supplied and made use of without toxic symptoms, although large doses were found to cause renal irritation. Blood serum contains practically 1 per cent protein, and ascitic fluid 0.17 to 1 per cent, hence in order to supply sufficient protein it would be necessary to give even on the basis of Chittenden's low estimate of 0.12 gm. nitrogen per kilo daily, 840 to 4,200 c.c. of fluid for a man weighing 70 kg., depending on whether blood serum or ascitic fluid was used, certainly too large an amount to be readily obtained or used on account of mechanical objections. At the same time, it is possible to use from 300 to 400 c.c. daily probably without detriment to the organism, although the urine should be watched for signs of renal irritation. In dogs, even large amounts were used and apparently utilized, although there was always a negative nitrogen balance in two- or three-day periods of from 0.04 to 4.35 gm. nitrogen; in starvation the balance being for two days, 3.83 gm. nitrogen daily(*l*).

When serum or ascitic fluid is aseptically drawn, it can be used safely; if there is any question it should be heated to 55° C., which makes it opalescent, but does not coagulate it.

Horse serum heated to 65° C. in amounts of 100 to 120 c.c. was used by Salter(*m*), who noted that the urinary nitrogen was increased. This, however, is not an homologous serum and could not be used for nutritional purposes without first testing the patient for serum reaction, and is not suitable for hypodermic feeding.

Fats.—Fat injections have been tried in various forms but too few accurate metabolic estimations have been carried out to place the matter on a firm footing. Von Leube used subcutaneous oil injections 20 to 30 gm. at a time two or three times daily, and concluded that the oil was absorbed and metabolized as evidenced by lowered excretion of nitrogen in the urine. Absorption is very slow, and care must be taken not to inject the oil into a vein which of course would result in fat embolism.

Mills(*n*), who has done much work on this, and presents the best historical résumé of the subject, finds that fats similar in composition to fats of the body are best absorbed, emulsions better than plain oils, the best being a 3 to 5 per cent emulsion of egg lecithin in sterile water. Sixty grams of oil may thus be given slowly. He also used oils of lard, cocoanut and peanut oil emulsified with egg lecithin, and proved that fats introduced subcutaneously may be burned directly, sparing body fat, and may be either retained in the body in their own form or may be reconstructed into body fat.

Lard, according to Winternitz, can be given by subcutaneous injection, but is of slight usefulness except in an emergency.

Carbohydrates.—The only form of carbohydrate which has been successfully used has been dextrose. Voit(*o*) in 1896 found he could inject a 10 per cent solution without producing glycosuria, although it was too painful a process, caused too much tissue infiltration and was not practical. Kausch used a 2 per cent solution, injecting as much as 1,000 c.c. In an 8 to 10 per cent solution it was promptly excreted in the urine, although it produced no renal irritation. It was also observed by him that the poorer the patient's nutrition, the better was the sugar borne. Gautier found he could use 60 to 80 gm. in 1,000 c.c. of sterile normal saline solution, and that it was well absorbed; but this furnishes only about 240 to 320 calories, which is not more than a fraction of the necessary amount. A four and one-half per cent solution of dextrose is isotonic with the blood, and would seem the best strength to use.

Salts and Water.—The hypodermic method of getting water and salts into the system has long been used with complete success and has formed one of the easiest and safest ways of supplying these necessary elements when the normal route is closed. This can be given as sterile normal saline solution (0.6 to 0.9 per cent) or in the following solution, which forms a more complete reproduction of the saline elements in normal serum:

Sodium Chlorid	0.9 gm.
Calcium Chlorid	0.026 "
Potassium Chlorid	0.01 "
Aq. destil	99.064 "

Taken then altogether, it can easily be seen that as yet the subcutaneous method of maintaining nutrition is of minor importance and practically about all that can be done is to supply a small amount of protein in the form of blood serum or ascitic fluid (with a little emulsified fat given separately?) and dextrose in a 4.5 per cent solution in normal saline. The serum or ascitic fluid may prove of benefit eventually in treating certain diseases, e. g., cholera where the loss of fluids and nitrogen is excessive, care being taken to rule out the presence of syphilis or tuberculosis in the donor before using either; but even here the intravenous route

is better and more satisfactory. It must also be said that for short periods the intravenous route is better for giving glucose solutions also.

Intravenous Feeding.—The intravenous method of giving medication for varying conditions has come into vogue more and more, and is now an established method of practice. The application of this principle to supplying nourishment to the body is of very recent date, and a field of usefulness has been opened that may be fruitful of very definite results.

There are certain dangers connected with this method that do not obtain in other forms of artificial feeding and must be taken into account. Embolism is a possibility, but is probably of slight moment with anything like surgical cleanliness and is certainly a rare occurrence in giving medication. Overfilling of the blood vessels is another potential danger, and with a weakened heart muscle must be kept in mind, and the amount injected into the vein carefully regulated as to speed of introduction and total quantity used.

Indications.—The chief indications for this form of feeding may be summoned up as follows: 1. When all other routes are closed. 2. In conditions of severe acidosis. 3. In severe acute infections. 4. To produce massive diuresis. The last three indications are to meet medical rather than nutritional demands.

Protein.—The use of protein by the intravenous route, except in the form of serum, is still in the experimental stage and no reference can be found in recent literature bearing on the subject. Woodyat reports that he and his collaborators have been doing experimental work with proteins but is not yet ready to publish it. It would seem a simple matter to supply protein in a limited way intravenously by using human serum, but the difficulty would naturally arise in securing a supply to carry on the food requirements. Horse serum could be used for a short time, provided the individual was not sensitive to it. The process is still in a speculative and experimental stage with as yet no definite solution of the problem of supplying easily the protein requirements of the body by this method.

Fat.—From what is known of fat embolism it would seem that the giving of fat by the intravenous route was pretty definitely precluded, and although a 3 per cent lard emulsion has been used experimentally in animals, it is not without danger and should not be used in man.

Carbohydrates.—Again, as in the rectal and subcutaneous methods of feeding, carbohydrate in the form of dextrose is the most easily used and readily absorbed and forms, so far, the only important constituent of this method of artificial nutrition.

Woodyat, Sansum and Wilder, by means of a special apparatus, described in the *Journal of Biological Chemistry,* tested glucose tolerance by intravenous injection, and showed that by delivering it at a uniform rate of speed in 10 to 50 per cent solutions, a rate closely corresponding to 0.85 gm. of glucose per kilo of body weight and hour of time, for from six to

twelve hours, it was possible to give such solutions without producing glycosuria or diuresis. The following conclusions were drawn from these experiments:

1. A man weighing 70 kg. may receive and utilize 63 gm. of glucose by vein per hour without glycosuria, which equals 252 calories per hour or 6,048 calories per day, which is about twice his resting requirements.

2. This is in accordance with Blumenthal's conclusions in animal experiment by repeated small doses.

3. These experiments discredit the idea that the glycogenic function of the liver is indispensable for the utilization of sugar.

4. The theory that any large amount of glucose given by vein always causes glycosuria and diuresis must be given up.

5. The tolerance limit of levulose was 0.15 gm. per kilo the hour; galactose about 0.1 gm.; lactose practically zero.

6. When glucose is given intravenously faster than 0.9 gm. per kg. the hour, glycosuria appears, then later, diuresis, these are all of practical importance.

7. If given faster than 0.85 gm. per kg. the hour, "the unburned glucose begins to accumulate in the tissues and pass out chiefly in the urine and carries water with it," extensive diuresis resulting.

To make 12.5 gm. glucose pass out of the body via the kidney at least 100 c.c. of water is necessary; if too much water is given, there is danger of mechanically stopping the heart.

In the practical application of these conclusions to intravenous feeding, it would seem unwise and unnecessary to try to supply the limit of the body tolerance 0.85 gm. per kg. the hour, and that the most that can be done is to furnish a fraction of this limit, enough to partially spare the protein destruction, and prevent marked acidosis. To furnish not over one-half the caloric needs of the body at rest, e. g., for a man of 70 kg., using an isotonic glucose solution (4.5 per cent), it would be necessary to give 305 gm. glucose in 24 hours, using 6,800 c.c. of the solution, altogether too large an amount even if divided up into two or three injections. If a 10 per cent solution were used, it would require 3,050 c.c., and if given at the rate of 63 gm. per hour, it would require 4.8 hours to give. This, of course, could be done, but could not be kept up for more than a few days (even dividing the dose into three of 1.6 hours for each dose) on account of the inability to use the veins over and over again. So far as using the special pump described by Woodyat goes, this would hardly be practical in humans, but the solution could be given from an irrigator kept warm by a jacket, and warming the solution just before it enters the vein by passing the tube under a hot water bottle, using about 180 drops per minute. The same rate of flow and temperature curve could be used as recommended in Kemp's table (see rectal feeding, p. 814). The solution in which the glucose is

dissolved should be a normal 0.9 per cent saline, freshly distilled and sterilized. This, of course, furnishes no protein and the patient would have to burn his own protein, although a certain amount would be spared on account of the glucose. Whether later it will be found possible to incorporate blood serum or some form of amino-acid compound to supply the protein of the diet must remain for future investigation. Intravenous feeding must at best be only for very temporary use in exceptional cases.

The use of glucose solutions for the other demands mentioned will be found under their appropriate heading in Diabetes Mellitus, Acute Infections, and Renal Disease, q. v.

Transfusion of Blood . . *George R. Minot and Arlie V. Bock*

Introduction—General Effects of Anemia on the Body—Beneficial Effects of Transfusion—The Effect upon the Oxygen Capacity of the Blood—The Effect upon the Blood Volume—The Effect upon the Factors of Coagulation—The Effect upon Blood Regeneration—The Effect upon Immune Bodies—The Effect upon the Basal and Nitrogen Metabolism—The Effect upon the More Immediate Symptomatology—Indications for Transfusion—Conditions in Which Transfusion is a Necessity—Conditions in Which Transfusion is Often Desirable—The Amount of Blood to be Transfused—The Choice of a Donor—Reactions from Transfusion—Reactions Due to Recognized Incompatibility—Reactions Not Due to Recognized Incompatibility—Methods of Transfusion.

Transfusion of Blood

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I. Introduction

Transfusion of blood is a standard therapeutic measure. Its usefulness has outgrown the older conception that it is only an emergency operation. Holtz has traced the history of transfusion back to Cardanus' work in 1556. The simplification of transfusion methods has made it possible for those not particularly trained in surgical technic to transfer blood from one individual to another, while the possibility of avoiding hemolysis by preliminary tests has eliminated the chief risk. In spite of these facts, the majority of physicians still regard transfusion as a formidable operation. It is our purpose here to discuss the transfusion of blood from different aspects, especial emphasis being placed upon the physiological principles that form the basis for its use in therapeutics.

II. General Effects of Anemia on the Body

In order to appreciate some of the effects of transfusion in cases of anemia it is desirable to consider briefly certain disturbances which occur when there is a diminished amount of circulating hemoglobin in the body. In general, it may be said that anemia impoverishes the functions of all the organs of the body and produces certain deleterious changes. Well known clinical manifestations indicate the existence of the condition. These vary according to the degree of the anemia, but they may include dyspnea, palpitation, gastro-intestinal disorders, disturbance of kidney function, symptoms referable to the central nervous system, and, in extreme cases, complete prostration may result. The latter condition is often regarded as cardiac failure, the underlying anemia having been overlooked.

Very little definite knowledge is at hand to show the relation of such clinical manifestations to altered function of the body. Strauss(*b*), quot-

ing the work of Von Noorden, Krause, Ribbert, and others, states that the fatty infiltration and degeneration of tissues occurring in chronic anemia is an indirect result of the low hemoglobin content of the blood. He assumes that the excessive effort of the tissue cells to procure oxygen from the anemic blood produces such an alteration in the cells as to predispose them to fatty infiltration. Until recently the only available metabolic observations in anemia were those made upon scattered cases by various observers, and those which concern the effect of acute hemorrhage in animals. No precise agreement in either series of observations is apparent. There often has been found in anemia of all types a negative nitrogen balance, usually not great. The notable exceptions to this finding occur in the work of Von Noorden, Goldschmidt, and his associates, Mosenthal(*d*) and Minot(*a*). The problem of nitrogen excretion after hemorrhage in normal animals is somewhat different, but Haskins and others have found an increase in protein metabolism which is only temporary.

Studies of basal metabolism in anemia have also shown great variations. Anemia does not necessarily result in a sluggish metabolism, since the demand for oxygen may be somewhat greater than in health. Meyer and DuBois determined the metabolism in five cases of pernicious anemia and found an increase of from 2 per cent to 33 per cent. Tompkins, Brittingham and Drinker have shown that the basal metabolism in anemia may vary within normal limits, or be above or below normal. Although they found no close parallelism between the degree of anemia and the basal metabolism, they concluded that the cases of anemia with acute symptoms have a high metabolism while the chronic cases have a diminished oxygen consumption. Zuntz(*b*) and his associates showed that muscles poorly supplied with oxygen are functionally less efficient. Accessory muscles are therefore called upon for the accomplishment of any task, as in respiration, thus increasing the demand for additional oxygen, a factor which may account for part of the increased metabolism in some cases, according to Meyer and DuBois. Lusk(*h*) expresses the view that the general oxidation of the body is normally maintained in anemia provided the anemia is not of extreme severity, and that lack of oxygen renders the anemic individual incapable of great muscular work without quick exhaustion.

In view of the fact that in anemia the body suffers from decreased function of many organs, and in view of the possibility of a normal or augmented metabolism in the presence of anemia, the question arises as to how the oxygen requirements of the body may be met. Certain phenomena may be mentioned which may for long periods of time partially compensate for the oxygen deficit. These are increased rate of blood flow, increased ventilation by the lungs, and increased utilization of oxygen in the blood. Often the immediate purpose of transfusion is to relieve the body of these excessive compensatory efforts and thus to restore normal function.

III. Beneficial Effects of Transfusion

Whatever the purpose for which transfusion may be done, there are various beneficial results to be obtained by the procedure which may be enumerated before a discussion of them is undertaken. They are as follows: 1. The effect upon the oxygen capacity of the blood. 2. The effect upon the blood volume. 3. The effect upon the factors of coagulation. 4. The effect upon blood regeneration. 5. The effect upon immune bodies. 6. The effect upon the basal and nitrogen metabolism. 7. The effect upon the more immediate symptomatology.

1. **The Effect upon the Oxygen Capacity of the Blood.**—One of the chief objects of transfusion is to increase the power of the recipient's blood to carry oxygen. In normal blood the total oxygen capacity which depends upon the hemoglobin content of the corpuscles is about 18.5 volumes per cent. After acute hemorrhage or in severe anemia this figure may be reduced to one-fourth or one-fifth of the normal, and in such conditions it is obvious that more hemoglobin must be introduced into the circulation in order to avoid oxygen starvation of the tissues. This can be done only by giving red corpuscles for which there is no known substitute.

In the resting normal individual the venous blood returns to the heart with a reserve oxygen supply of 12 to 14 volumes per cent. In a state of grave anemia, however, as Lundsgaard(*e*) has pointed out, the tissues may demand the last residuum of available oxygen from the blood, just as readily as the first part, and the blood may return to the heart in a nearly completely asphyxiated state. At the present time there are no figures showing complete asphyxiation of venous blood in man, but the blood of many cases of severe anemia closely approximates this condition. Pflüger and Voit also showed that the demand of the tissues for oxygen was independent of the supply. The reduction of the oxygen combining power of the blood may be so great in extent that the ordinary compensatory factors may not be sufficient to maintain the internal respiration of the body even in a completely resting individual. A condition of this nature is perhaps most often seen in pernicious anemia in which the occurrence of great prostration and tissue changes of serious extent form a familiar clinical picture. What may be immediately accomplished in such a patient is illustrated in Table I, in which is presented the data of a case before and after transfusion of 600 c.c. of blood, together with the oxygen figures for the blood of a normal individual for comparison.

In contrast to a normal oxygen reserve of 12 to 14 volumes per cent, this patient had less than two volumes per cent which accounts for his complete physical disability. The longer an individual remains in such a condition the greater the irreparable damage to body structure. Thus if

TABLE I

Diagnosis	Red Count in Millions	Pulse Rate per Minute	Blood Press. in mm. Hg	Arterial Blood		Venous Blood		Hemoglobin %
				Oxygen Cap. in Vol. %	Oxygen Cont. in Vol. %	Oxygen Cap. in Vol. %	Oxygen Cont. in Vol. %	
Pernicious Anemia.....	0.82	112	100/50	4.42	4.20	4.42	1.95	23.8
After Transfusion.....	1.5	100	110/50	...	...	6.67	2.45	36.
A normal man.....	4.5	72	128/80	19.6	18.5	19.6	11.96	106.

transfusion is decided upon in cases of chronic anemia the procedure should not be postponed for weeks to see first if the patient will not regenerate some of his own blood. After this case had received 600 c.c. of blood the increase in hemoglobin was equal to 50 per cent of the amount in the circulation before transfusion. Even so, the total hemoglobin remains only one-third of the normal. Though this amount of hemoglobin is insufficient to enable the organs of the body to function well, it permits them to act distinctly better than with the amount of hemoglobin present before transfusion. In fact, it is rather striking that a slight elevation of the hemoglobin level will often largely remove the symptoms of anemia.

The actual increase per c.c. of blood in the number of red corpuscles after transfusion depends upon such factors as the amount of blood transfused, the amount of plasma in the recipient's circulation, the degree of anemia present and certain unknown factors among which may be a possible redistribution of blood, as Huck has suggested. When about 600 c.c. of blood is given, the usual increase in the number of corpuscles is from 200,000 to 700,000 per c.mm., and the hemoglobin is increased within a range of 5 to 20 per cent. There may not be a very close relationship between the increase in the number of corpuscles and the percentage increase in hemoglobin. Rarely, after transfusion, no increase in red corpuscles can be demonstrated by counts.

The beneficial effect of the transfused red cells in increasing the oxygen carrying capacity of the blood must be regarded as only temporary. This is because they do not remain indefinitely in the circulation of the recipient. According to the work of Ashby the life of transfused corpuscles may be as long as thirty days and under certain conditions even much longer. Previous work has suggested that 10 per cent of the red corpuscles are destroyed daily. Though the transfused red cells themselves increase temporarily the oxygen carrying capacity, transfusion will often tide the patient over a period of time until he can furnish enough cells to serve satisfactorily the functions of the body.

In considering the necessity for transfusion, emphasis usually is to be placed upon the hemoglobin content of the blood. Fluid substitutes for

blood have their uses but they cannot take the place of blood if increased oxygen carrying power is needed.

2. **The Effect upon the Blood Volume.**—In most conditions for which transfusion is indicated, a diminished volume of circulating blood usually exists, either by reason of a mechanical reduction in the whole blood, as after acute hemorrhage, or on account of a diminished content of red corpuscles which is associated with most types of anemia. Reduction of the plasma volume may occur following blood loss, and in other anemias when the hemoglobin is below 30 per cent. Transfusion of blood after a severe hemorrhage may help to restore the plasma volume to about its normal figure but the total blood volume may not be regained except through regeneration of corpuscles unless it is made up by repeated transfusions. Hypertransfusion should be avoided because of the possibility of bone marrow depression, as demonstrated experimentally by Robertson(*c*).

In chronic anemia, in contrast to acute anemia due to blood loss the volume of the plasma is usually not abnormal if the patient has a normal fluid intake. When transfusion is undertaken for such a condition, the only gain in total blood volume is due to the addition of corpuscles. Under such a circumstance the plasma of the transfused blood rapidly leaves the circulation for the tissues. This consideration is an important one, since it shows that alterations in the blood volume in anemia are almost wholly dependent upon variations in the total mass of corpuscles, as discussed by Bock. There is no method of increasing the total blood volume in chronic anemia except by the addition of corpuscles.

3. **The Effect upon the Factors of Coagulation.**—In the various forms of purpura hemorrhagica there occurs a deficiency in the number of blood platelets which is associated with the pathologic hemorrhage frequently encountered in these cases. In hemophilia, as Minot and Lee(*a*) have shown, there occurs a qualitative deficiency of the blood platelets. In other conditions in which pathologic hemorrhage occurs, there are often unknown alterations in the physical chemistry of the blood which interfere with normal clot formation. This may be due to an upsetting of the balance of prothrombin and antithrombin as, for example, by a decrease of the former or increase of the latter substance, or there may be a deficiency of fibrinogen or some other not well recognized alteration. The only truly efficient way of remedying a defect in one or more of the factors that promote clotting, is by transfusion of normal blood which contains all of the factors. It is to be recognized that serious bleeding associated with a deficiency in the numbers of platelets, does not occur until these elements have been reduced from their normal number of about 300,000 to 60,000 per c.mm. or below. If a liter of normal blood is transfused the platelets will be increased in the recipient's blood by about 70,000 per c.mm. Thus, when transfusion is necessary to stop bleeding due to a deficiency of platelets, a large amount of blood should be given

in order to restore a sufficient number of platelets to prevent spontaneous bleeding. It is, however, probable that other elements in the blood assist to check a hemorrhage particularly associated with a deficiency or a defect in the platelets.

The duration of the life of the platelets is but a few days in contrast to the longer life of the red corpuscles. Thus if a patient does not make up some of his platelet deficiency within 3 to 5 days following a transfusion for such a defect, one must anticipate a recurrence of the spontaneous hemorrhage. Hence further transfusion will be necessary if it is desired to continue to check the bleeding. In hemophilia, in contrast to the various forms of purpura hemorrhagica, hemorrhage is not spontaneous but follows as a result of trauma, though this may be exceedingly slight. In order to check a severe hemorrhage in hemophilia, enough blood should be given to reduce the clotting time of the patient's blood to approximately normal. By means of such a procedure, hemorrhage is checked and thus the bleeding point allowed to close. Later, as the transfused platelets disappear from the circulation, the clotting time of the hemophiliac's blood again becomes abnormally prolonged. Hemorrhage does not recur unless the external or internal wound has not healed sufficiently. Hemorrhage will of course recur when there is sufficient further trauma. Transfusion may also be undertaken in hemophilia to prevent bleeding when operation has to be performed. Under such conditions it may be desirable to remove some blood before the normal blood is injected.

In Table II is shown the effect of transfusion on the blood of a hemophiliac in whom rather severe bleeding was to be anticipated from the extraction of teeth, if no normal blood had been given.

The foreign blood, with its normal platelets, held the clotting time of the patient's blood, with its qualitatively defective platelets, close to normal for enough time to permit primary healing of the wound.

In hemorrhagic disease of the newborn, the effect of transfusion is, in a very high percentage of the cases, very striking, for here it seems that normal blood is capable of doing more than tiding a patient over a critical period. Following adequate transfusion in such cases there nearly always occurs a permanent correction of the blood defect which is associated with a prolonged coagulation time and prothrombin time. To accomplish this result it may be necessary to give several doses of blood, but frequently 40 c.c. suffices.

In other conditions in which pathologic hemorrhage occurs due to recognized or unrecognized blood defect, the principle outlined above applies, namely, that if transfusion is to be used, enough blood, which will furnish all the factors for coagulation, must be given to accomplish the desired result.

4. **The Effect upon Blood Regeneration.**—When the bone marrow is functioning deficiently, an increase in its regenerative activity often

TABLE II

Date	Coagulation Time in Minutes [1]	Transfusion	Remarks
May 1 10 A.M.	60	.....	Slight bleeding from about carious teeth
10.30 A.M.	..	1000 c.c.	
11.30 A.M.	10	.....	Teeth removed—no abnormal bleeding
May 2	15	.....	No bleeding
May 3 10 A.M.	20	.....	Slight bleeding
11 A.M.	..	500 c.c.	
11.30 A.M.	8	.....	No bleeding
May 4	15	.....	No bleeding
May 5	20	.....	No bleeding
May 6	30	.....	No bleeding
May 7	50	.....	No bleeding
May 8	65	.....	No bleeding

[1] Time required for 1.5 c.c. of venous blood to clot in a test tube 8 mm. in diameter. Upper limits of normal 15 min.

occurs following transfusion. This may be due to a direct or indirect effect of the transfused blood. Increased bone marrow activity may be manifested not only by increases of young red cells but increases also of platelets and marrow white cells above a level due to the transfused blood. In other instances, when the regeneration is not so rapid, significant increases of young red cells do not occur, but the platelets and marrow white cells remain at a higher level than before transfusion. If a suitable formation occurs the count of the red cells remains elevated and increases while the transfused cells gradually cease to exist in the circulation. Such a picture indicates that the bone marrow elements are being delivered into the circulation at a desirable rate.

Alteration in the white count following transfusion may be associated with a mechanical redistribution of the blood in the same manner as the red cells. Thus, elevation of the white count does not necessarily indicate a general increase of bone marrow activity. A sharp leukocytosis following transfusion may be only a further manifestation of a reaction due to the foreign blood, as described on page 840, rather than a sign of general marrow activity. Still the degree of leukocytosis indicates roughly the ability of the marrow to produce blood even though the transfusion may not be followed by an increase of blood production. Alterations in the platelets may occur after transfusion in a similar manner.

However, if both the platelets and marrow white cells increase in number and remain elevated after transfusion, these rises should be interpreted as evidence of increased marrow activity. With increased regeneration the platelets usually begin to increase in number slightly later than the white cells. With an orderly increased activity of the marrow such as may occur in pernicious anemia, the reticulated red cells (young cells) begin to increase still later—that is, in about three to five days.

The response of normal bone marrow to the stimulus of hemorrhage is more rapid and proceeds more uniformly with respect to all of the blood elements than may be seen after transfusion in cases having pathological bone marrow. There may occur with regeneration of blood, with or without transfusion, a distinct qualitative change in the process of regeneration such as a disproportionate output of platelets, or of young red corpuscles, in relation to the other elements produced by the bone marrow. If the marrow is aplastic the response to transfusion may be very feeble or more often does not occur. Distinct inactivity or depression of the bone marrow following transfusion is a bad prognostic sign. Likewise the presence in the peripheral blood of very large numbers of immature marrow cells of the red and white series is unfavorable and indicates what may be termed a dissolution of the marrow. For a further discussion of the question of bone marrow activity, reference may be made to the work of Drinker, Vogel and McCurdy, and Minot and Lee.

5. **The Effect upon Immune Bodies.**—Theoretical considerations have led to the use of transfusion for the transfer from one individual to another of immune bodies, particularly for the treatment of disease. Experience up to the present is variable in character and, for the most part, disappointing.

In sepsis the supportive effect of fresh blood has long been thought to be beneficial, but in practice little good has been accomplished by such therapy, probably because normal blood has less bactericidal power than the blood of the patient. Wright and Colebrook have recently suggested a method of "immuno-transfusion" for cases of sepsis, in which the blood to be transfused may be rendered bactericidal in vitro, and then injected into the circulation of the patient. The vaccine used for this purpose need not be specific. The blood transfused in a case reported by Wright and Colebrook was thus immunized against the patient's streptococcus; the protective action of the serum against the patient's organism was previously demonstrated by a simple laboratory study. A cure resulted in this case in which operative and other therapeutic measures had failed.

6. **The Effect upon the Basal and Nitrogen Metabolism.**—Transfusion of blood in cases of anemia, according to Tompkins, Brittingham and Drinker reduces the basal metabolism to a normal or diminished level. They suggest that the basal metabolism may serve as a guide in knowing

when to push transfusion in the treatment of anemia, and when little may be expected from the procedure. For example, if the metabolism is minus 10, only temporary comfort to the patient is to be expected. If the result is plus 10 more will be accomplished by transfusion. Transfusion provides relief for certain compensatory phenomena such as increased pulse rate and increased ventilation of the lungs, but the demand of the tissues for increased oxygen may continue for days after the transfusion. Transfusion is regarded by these authors as a measure by which early cases of pernicious anemia may be assisted toward a remission. Studies at the Massachusetts General Hospital, yet incomplete, tend to show that the basal metabolism is not always indicative of what transfusion will accomplish in anemia.

Little is known as to the effect of transfusion upon nitrogen metabolism. Mosenthal(*d*) found a lowered nitrogen balance after transfusion, owing to the output in the urine of the nitrogen contained in the transfused blood. In dogs, Haskins(*a*) found that transfusion after hemorrhage does not prevent the destruction of protein which occurs as a result of hemorrhage.

7. **The Effect upon the More Immediate Symptomatology.**—Symptomatic improvement following transfusion depends not only upon the cause of the anemia but also upon the state of the patient. The greatest clinical change is seen in patients transfused after sudden loss of much blood. The usual signs of restlessness, rapid pulse, increased respiration and sweating, are improved at once or entirely relieved. A general sense of well being is substituted for a state of anxiety, and a condition of doubtful outcome may be changed at once to one having a favorable prognosis. The improvement is due to a number of complex factors, chief among which is the increased efficiency of the circulation as manifested by higher blood pressure in certain cases, slower pulse rate, and increased oxygen carrying power of the blood.

The more immediate symptomatic improvement in chronic anemia is *not* so pronounced, owing to structural changes in the body and to the probable persistence of the cause of the anemia, toxic or otherwise.

Weakness, palpitation, dyspnea, and visual and auditory disturbances are often relieved. If fever is present due to the blood condition, the temperature may subside after transfusion. Improvement of appetite and diminution of gastrointestinal symptoms frequently occur shortly after transfusion, especially in states of chronic anemia. Although achylia may persist in pernicious anemia the stomach distress present before transfusion may entirely disappear afterward. Troublesome diarrhea occasionally met with in pernicious anemia may also be controlled. It has been shown that the kidney function is deficient in chronic anemia, and, among other benefits that result from transfusion is improvement in the functional state of the kidneys.

IV. Indications for Transfusion

No detailed account of all of the conditions for which transfusion is indicated will be undertaken here. In a general way they belong to two groups, namely, conditions in which transfusion is an absolute necessity in order to save life and conditions in which the procedure may be desirable either for the comfort of the patient or to shorten convalescence.

1. **Conditions in Which Transfusion is a Necessity.**—The usual conditions in which transfusion may be obligatory in order to save life are hemorrhage and shock. Since moderate or severe hemorrhage is always accompanied by a state of shock, these two conditions may present the same indications for treatment. They have in common diminished blood volume and low blood pressure, both of which may be corrected, at least in part, by transfusion. In the case of hemorrhage, danger to life lies not so much in the extent of hemorrhage as in sudden loss of blood. The latter may result in a rapid fall of blood pressure to a dangerous level, a state in which the tissues of the body are deprived of oxygen owing to the failure of the circulation. Keith has shown that the blood volume in shock, not complicated by hemorrhage, may be diminished to the same extent as in hemorrhage. In such a condition the body may not survive for more than a brief period unless energetic measures are taken to increase the volume of the circulating blood, which in turn reacts favorably upon the blood pressure. Fluid substitutes for blood, such as gum-saline, may serve to restore the circulation and may be used instead of blood when the blood loss has not been too great. In shock gum-saline is highly useful if it is used soon after the advent of the condition. However, if such a fluid is not available, normal salt solution may temporarily tide a patient over a brief period of time until transfusion can be carried out.

The criteria upon which to judge the condition of the patient are blood pressure readings, hemoglobin determinations and pulse rate, as has been discussed by Robertson and Bock. A very low systolic blood pressure, 70 mm. of mercury for example, after acute hemorrhage, or in shock, usually means a great diminution in blood volume. Subsequent blood pressure determinations are important to note whether the reaction of the patient is favorable or not. For example, a rising blood pressure is a good prognostic sign. A single hemoglobin estimation, especially if made soon after hemorrhage has occurred, is of little significance. It is important to know whether subsequent hemoglobin readings at hour intervals are the same or steadily becoming lower. A flow of fluids from the tissues to the circulation, or internal transfusion, as Gesell has called it, will dilute the hemoglobin, and if this does not fall below 30 per cent, transfusion is not urgent though it may be advised. Cases of hemorrhage and shock in which the hemoglobin remains at a stationary figure for sev-

eral hours are almost always fatal, even with repeated transfusions. Large amounts of fluids administered by the alimentary tract may often accomplish the purpose for which transfusion or infusion seems indicated.

No absolute indication for transfusion exists so far as oxygen need is concerned, as long as the hemoglobin remains above 30 per cent. There is abundant evidence to show that animals, after bleeding to as low as 25 per cent of hemoglobin, will survive providing the fluid volume of the blood is maintained by intravenous injection of fluid substitutes for blood. In case the hemoglobin is below 30 per cent transfusion should be looked upon as a necessity and not as a matter of choice. Life itself may be immediately endangered, other things being equal, only when the blood contains less than about 30 per cent of hemoglobin.

As has been mentioned, transfusion may be necessary to control hemorrhage due to pathological blood defects such as occur in hemophilia, hemorrhagic disease of the new born, and other hemorrhagic conditions. It is reiterated here that it may be necessary to transfuse more than once to control hemorrhage of this type. Often in hemorrhagic conditions, transfusions also must be used in a preventive manner when operation becomes necessary.

2. **Conditions in Which Transfusion is Often Desirable.**—In the group of conditions now to be discussed transfusion of blood may be done to improve the general state of the patient though the procedure may not be a life-saving one. The articles by Pemberton, Garbat, McClure and Dunn, Lewisohn, Lindeman, Ottenberg and Libman, Bernheim, and Minot, among many others, consider this aspect of transfusion.

Transfusion in *pernicious anemia* has been discussed by Anders, Minot and Lee(*b*), and many others. It is generally agreed that transfusion in this disease helps to bring about remissions which probably would not otherwise occur. It appears to make remissions about 10 per cent and perhaps 20 per cent more frequent. It undoubtedly often adds greatly to the comfort of the patient. While remissions may be favored by transfusion, the natural course of the disease is not altered by such treatment.

Transfusion probably should be employed before the stage of great anemia and prostration has developed. The gradual failure of an adequate oxygen supply to the tissues is always critical because of the transformation of normal tissue to fat and water. Good results cannot be expected from any measure of therapy after such changes have occurred in the body. The value of transfusion in pernicious anemia at present is based for the most part upon its use in the treatment of cases in the stage of prostration due to such tissue changes. It is important that the diagnosis of pernicious anemia should be made early, and the cases transfused while the hemoglobin is still at a relatively high level in order to attempt to forestall the inevitable results of anemia. A detailed discussion of transfusion in this disease cannot be entered into here, as it is not our

purpose to discuss the treatment of pernicious anemia. One must consider the probability of remission as told by the history of the case, the character of the blood, etc., as well as the desires of the patient and his family when considering transfusion in this disease.

In other forms of *chronic hemolytic anemia* transfusion may be used similarly as in pernicious anemia. However, it is possible that in a case with increased blood destruction transfused corpuscles may perhaps remain in the circulation a shorter time than when a normal amount of hemolysis is occurring. For this reason, among others, in some forms of hemolytic anemia, such as chronic hemolytic jaundice, splenectomy is the best treatment and transfusion then may be used to improve the condition of the patient for operation.

In anemia from blood loss both acute and, particularly from chronic types, in which no emergency exists for transfusion, remarkable results may follow the use of this therapy. In addition to an increased output of corpuscles from the marrow, a definite permanent alteration of the color index of the corpuscles has been noted, in that the hemoglobin content per corpuscle seems definitely increased. In such cases transfusion restores the patient to health considerably sooner than with any other method of therapy. In cases of chronic anemia due to blood loss, when the bleeding has been stopped, the marrow may regenerate very sluggishly. Transfusion enables such patients, who may be chronic invalids, to regenerate blood and regain health often months earlier than without such treatment.

Single and often repeated transfusion is also of value in aiding a return to normal in *other forms of chronic anemia,* particularly if the cause has been removed, or if it is anticipated that transfusion will diminish the activity of the cause. A striking example of the effect of many transfusions, when the cause of anemia has been removed, is seen in severe *benzol poisoning.* This poison tends to produce aplasia of the marrow and the resulting clinical and blood picture is that of aplastic anemia with secondary purpura hemorrhagica. When the influence of the poison is removed the blood may return to normal. However, in the severe cases the trap seems to be sprung so far that the marrow is unable to regenerate at the moment enough blood to maintain life. In some such cases repeated transfusion performed about as often as bleeding recurs, permits the patient to live during the time the marrow regenerates to a point at which it can supply sufficient blood elements to maintain satisfactorily the needs of the body.

In idiopathic aplastic anemia transfusion appears to result in only temporary benefit, for, unlike the cases of benzol poisoning, the unknown cause is not removed.

Besides the use of transfusion to stop hemorrhage and to prevent its occurrence at operation in a patient having a hemorrhagic disease, repeated transfusions may be used in certain conditions to accomplish the same

results as in benzol poisoning. Cases of acute *idiopathic purpura hemorrhagica* best illustrate this. Here repeated transfusion checks hemorrhages and supplies red corpuscles, and in so doing the transfused corpuscles may keep the individual alive until the unknown cause diminishes so that the platelets can return to normal as sometimes occurs. In cases of secondary purpura hemorrhagica, and other hemorrhagic states, where the cause cannot be removed, no real benefit can be anticipated from repeated transfusion.

Transfusion also finds valuable use in improving the condition of the *patient with anemia before operation* is undertaken, even though the anemia is not great. Ottenberg and Libman, among others, have commented on the value of transfusion preparatory to operative procedures.

Transfusion has been used to combat *sepsis* and *toxemias* such as eclampsia, but no definite beneficial results have been obtained.

From time to time transfusions have been reported for the cure of *carbon monoxid poisoning,* but there is almost no evidence forthcoming to show that transfusion is beneficial in this condition. Crile and Lenhart found that transfusion was the most efficient therapy in the restoration of dogs overcome by carbon monoxid gas, but clinical results have not met with the same success. Henderson has summarized our present knowledge concerning the effects of carbon monoxid as follows: It is a physiologically harmless gas except in its affinity for hemoglobin, and its toxic effects are entirely due to the inability of the blood combined with carbon monoxid to transport oxygen. Hemoglobin has a very great affinity for carbon monoxid, but the combination is not a permanent one and is rapidly broken up in the presence of oxygen or pure air. Injury resulting from this gas occurs during the time in which the patient breathed carbon monoxid. When placed in an atmosphere of pure air almost all of the carbon monoxid is eliminated from the body within a period of one to three hours, if recovery is to occur. Transfusion cannot repair the injury caused by this gas. The treatment consists mainly in fresh air and symptomatic measures. However, in some instances transfusion may be very beneficial, as suggested by Lindeman's case.

In other conditions, such as *nitrobenzene poisoning,* there occur other forms of altered hemoglobin than CO-hemoglobin, namely, methemoglobin and NO-hemoglobin, which prevent oxygen from being transported. The amount of these abnormal forms of hemoglobin may be so great that extreme cyanosis is present and less than 30 per cent of oxyhemoglobin remains. Under such conditions transfusion may be required. Usually with the formation of altered hemoglobin the patient's condition is not severe enough to require transfusion. Cases of nitrobenzene poisoning show a surprising tendency toward spontaneous recovery when the source of the poisoning is removed, as is the case in CO poisoning. However, we have seen death occur from the effects of this substance and others,

as Donavon, have reported the same result. Two cases of nitrobenzene poisoning that we have personally observed had their oxyhemoglobin reduced to 30 per cent and 35 per cent, respectively. Both recovered with transfusion.

V. The Amount of Blood to be Transfused

It is generally agreed that a donor may give blood up to one quarter of his blood volume without serious discomfort. A man weighing 70 kilograms has a blood volume of about 5,500 c.c., hence blood may be taken from him for purposes of transfusion up to about 1,300 c.c. It is seldom necessary to use such a mass of blood for transfusion, but it may be helpful to have in mind the limit of safety for the donor. This limit varies directly with the body weight.

What constitutes a proper amount of blood to be given for the different conditions in which transfusion is indicated has been suggested by various authors as a result of clinical experience. It has not been possible to make definite quantitative measurements of the various factors involved, and therefore only a general statement can be made with reference to this important subject. In every instance the weight of the patient to be transfused should be considered in order to avoid hypertransfusion. A normal individual has a volume of blood equal to 80 to 85 c.c. per kilogram of weight. A patient weighing 70 kilograms, with severe anemia, may have his blood volume reduced to 50 c.c. per kilogram, representing a reduction in blood volume of approximately 40 per cent. It would be futile to attempt to restore the normal blood volume by means of transfusion in such a case and fortunately this is never necessary. On the other hand, if repeated transfusions are done at intervals of a few days to control hemorrhage, as in hemophilia, hypertransfusion causing polycythemia should be avoided.

In the routine use of transfusion, owing to the great elasticity of the vascular bed, hypertransfusion seldom occurs. It is manifested chiefly by cough, by pain in the back, and, in rare instances, pulmonary edema may develop, as Unger has recently described. These symptoms may occur regardless of the rate at which blood is transfused. It is probable that the same symptoms might be produced by a relative hypertransfusion, that is, by the introduction of a large amount of blood into the circulation of a patient having a greatly reduced blood volume, such symptoms being due to temporary embarrassment of the circulation.

When transfusion is indicated for loss of hemoglobin after hemorrhage, a large transfusion, 1,000 c.c., may be necessary. In chronic anemic conditions smaller amounts of blood, 300 to 750 c.c., may serve as well as larger amounts. In chronic anemia there is some evidence to show that a small quantity of blood, repeated within a few days, may be more bene-

ficial than a single transfusion of a large amount. As an explanation for the fact, it has been suggested that the bone marrow reacts better following a small than a large transfusion. When transfusion is indicated in hemorrhagic conditions enough blood should be given to stop the hemorrhage. This is usually a large amount rather than a small one.

VI. The Choice of a Donor

The donor must be in good health. He should have a negative Wassermann reaction, and should be able to provide the requisite amount of blood desired for the particular case. It must be realized that the amount the donor can spare and the amount the patient may receive should be considered in relation to the body weight of each. A donation of 500 c.c. of blood from a donor weighing 50 kilograms is equivalent to a donation of 800 c.c. from a man weighing 80 kilograms.

The blood of the donor should be compatible with the blood of the patient, that is, the red corpuscles of the donor's blood should not be agglutinated by the serum of the patient. It is also desirable, but not as important, as explained below, that the serum of the donor should not agglutinate the patient's red cells. The test for compatibility is a simple one and no transfusion should be done, except in an emergency of an extreme nature, unless the donor's blood is shown to be suitable for the patient. It is important not only to avoid iso-agglutination, but also iso-hemolysis, which is a greater danger than iso-agglutination. Iso-hemolysins are found in many but not all adults in whom iso-agglutinins are present, but they are not present if iso-agglutinins are absent. This is convenient, because by tests for agglutination, one may rule out the possibility of iso-hemolysis occurring as well as iso-agglutination. The results of iso-agglutination tests obtained in vitro, if carefully performed, are a reliable index as to what will occur in vivo, so far as iso-agglutination and iso-hemolysis are concerned.

Through the work of Moss and Jansky, it is now known that the blood of each adult falls into one of four definite groups, as shown by the agglutination reactions of the red corpuscles and serum.

These groups are shown in Table III.

The blood of each group is absolutely compatible within the group; that is, no iso-agglutination or iso-hemolysis will occur when two bloods of the same group are mixed in vivo or vitro. The group characteristic may not be fully established at birth. If it is not, in most cases it is established during the first year of life. Once established, the group of each human being appears never to alter in health or disease. Studies on the iso-hemolysins and iso-agglutinins of infants are reported in the recent papers of Happ and Basil B. Jones.

TABLE III

		Red Corpuscles of Group [1]			
		1	2	3	4
Serum of	Group 1	0	0	0	0
	" 2	+	0	+	0
	" 3	+	+	0	0
	" 4	+	+	+	0
Per cent of frequency		5	40	10	45

0 = no agglutination + = agglutination

[1] The classification given here and referred to in the text is that given by Moss. Since this paper was originally sent to the press, it has been officially recommended (Jour. A. M. A., 1921, 76, 130.) that on the basis of priority the Jansky classification be adopted, in spite of the fact that the Moss classification has been in wide use in America and Europe. The Jansky classification is considered identical to Moss' except that groups 1 and 4 are interchanged. However, it is not known that Moss' groups 2 and 3 are actually identical to Jansky's. This is because there is no evidence that anyone has compared the blood of an individual belonging to group 2 or 3 as determined by known sera or cells originating from Moss against the blood of individuals classed by Jansky as group 2 or 3.

When a donor is to be tested for the compatibility of his blood with that of a patient, it can be accomplished in two ways. The first one involves testing directly the donor's cells and the patient's serum for agglutination, and the patient's cells and the donor's serum. If no agglutination occurs with both of these combinations of cells and serum, it indicates that the two individuals belong to the same group, thus their bloods are compatible. If either of the tests is positive it indicates that the individuals belong to different groups. These tests do not tell us to what group the individual belongs. This is of no real consequence, for our object is only to transfer blood which is compatible. The second way in which one may determine whether a donor's blood is compatible with that of a patient is to determine the blood group of each. This may be done by testing the blood of each (either cells or serum) against bloods (either serum or cells) whose groups are known. If both belong to the same group, their blood is compatible. The blood of individuals of a certain group may be given to those of another group, as is referred to later, even when the subjects belong to different groups and their bloods are not strictly compatible.

The determination of the blood group of a patient and prospective donor frequently simplifies the selection of a donor in that the blood tests may be carried out at different times and in different places. Furthermore, blood only need be taken once from the patient. However, in order to control all possible errors, it is distinctly advisable just before each and every transfusion to test the recipient's serum against the cells of the selected donor.

The simplest way to determine to what group a given blood belongs is to test its cells against the sera of groups 2 and 3. The reason why one may determine the group by these two agglutination tests is because, as will be seen by reference to Table III, there are but four possible combinations of positive and negative reactions of unknown cells with known sera of groups 2 and 3. These four different combinations, one for each of the four groups, allow identification of unknown cells by the presence or absence of their agglutination by groups 2 and 3 sera. It serves as an excellent control if when the group is determined a test is made between the unknown cells and group 4 serum, in addition to groups 2 and 3 sera.

While it is always advisable to choose a donor who belongs to the same blood group as that of the patient, this is by no means always necessary. This is because, owing to certain protective mechanisms associated with a preponderating blood whose cells can be agglutinated by other sera, it is possible to give plasma which can in vitro agglutinate and hemolyze the cells of such blood. However, in the body, the blood of the recipient will prevent agglutination or hemolysis of its cells by the donor's plasma if the transfusion is given under suitable conditions and in at least the usual amounts. One can never give, without serious risks, red cells that can be agglutinated by the patient's plasma, which is under usual conditions the preponderating plasma following transfusion. Consequently, a group 4 donor may be regarded as a universal donor, since his cells cannot be agglutinated by any plasma, and a member of group 1 can be regarded as a universal recipient since his plasma can agglutinate the cells of no other group. It is, as stated, desirable to transfuse blood within the same group, yet as a practical measure it has been demonstrated repeatedly that blood of group 4 can be utilized for transfusion in any one of the four groups.

The practical advantage of regarding a member of group 4 as a universal donor is, of course, obvious. It merely requires the testing of a donor and does not require the testing of a patient. This enables one to have a supply of group 4 donors on hand for possible emergency transfusions. With the presence of a combination of a great reduction of blood volume, a marked reduction of red cells, an anticipated transfusion of a large amount of blood, and a strong iso-hemolysin in the donor's blood, it is unwise to transfuse from a group 4 donor into a recipient of another group. Clinical experience justifies this exception to the rule of the use of group 4 individuals as universal donors, when it is difficult to obtain a donor of the same group as that of the patient. It is, however, more desirable under any circumstances to use a group 4 donor for an individual of another group than one thought to belong to the same group as the patient, but whose group designation is not clear cut. This is particularly true when dealing with groups 1 and 3 patients whose iso-agglutinins and red cell receptors are apt to be of a weaker nature than

those of groups 2 and 4. For a more detailed discussion regarding the iso-agglutinins, iso-hemolysins and the selection of donors, the reader is referred to the references cited above and to those by Brem, Minot(*b*), Coca, Vincent(*b*), Sanford, Rous and Turner, Karsner(*b*), Karsner and Koeckert, Clough and Richter.

It is not the purpose of this article to discuss technic, but it seems desirable briefly to summarize a suitable method for performing these agglutination tests. This summary is essentially the same as that previously given by Minot and Lee.

In order to make a test between serum (fresh or stock) and the red cells, the following simple procedure with chemically clean glassware will usually suffice. A suspension of cells (about 5 per cent) is obtained by the addition of 3 to 5 drops of blood to about 2 c.c. of 1 per cent solution of sodium citrate in 0.9 per cent sodium chlorid solution. These cells need not be washed. A drop of the red cell suspension is mixed with a drop of serum. It is important to make the mixture complete. This may be done upon a glass slide with a cover glass put over the mixture. The cover glass should always be raised and the cells and serum remixed several times before a negative reading is made. A hanging drop preparation permits neater technic and avoids drying. The test often may be read macroscopically, but should always be read microscopically, in order to avoid any possible errors except when it is rapidly and undoubtedly positive. In order to guard against possible errors, it is always wise to allow the mixture of cells and serum to remain for at least 30 minutes, preferable in the incubator. While there are few opportunities for confusion in this simple test, nevertheless the penalty of transfusion of incompatible blood may be so great that every reasonable care should be given to the performance of the test. Confusion may be caused by weak agglutination. It is always possible by employing different amounts of cells and serum, by incubating the mixture for some hours and by thoroughly washing the red cells, to decide the problem of doubtful reactions. However, if by the method described the reaction is not clear and perfectly definite, the test must be repeated and perhaps amplified. A safe rule is never to regard a reaction in which there is any doubt as negative. Rouleaux formation may be easily demonstrated as quite different from agglutination. Confusion may be caused by atypical agglutinations, that are very rarely intense, due to auto-agglutination and allied phenomena which are little understood. Stock sera for determining to what group a human being belongs will keep many months and even years if sterile, carefully sealed and in the ice-box. Stock sera have an advantage over fresh sera in that they are less liable than fresh sera to produce reactions with red cells, which may be confused with iso-agglutination. It may be again emphasized that when carefully done the reaction of agglutination is in a very large proportion of cases clear and

definite. In practice it is always expedient to discard as a donor one whose blood causes any doubt about his group or about the reaction of his cells with the patient's serum.

VII. Reactions from Transfusion

Previously, the beneficial effects of transfusion have been discussed. It is now necessary to point out the harmful effects which may result from this procedure. If a donor is used who is not healthy, syphilis, malaria and other diseases may be transferred to the patient. Hypertransfusion has been previously referred to and can always be avoided. Reactions due to incompatibility of blood, as shown in vitro, may occur if improper tests are made. The deleterious effects of transfusions done with completely proper technic are those in the nature of a reaction from some unknown alteration in the transfused blood, and, in some instances, dependent upon the state of the patient. Such reactions are very rarely serious.

1. **Reactions Due to Recognized Incompatibility.**—Reactions resulting from the transfer of blood incompatible with that of the patient's, in that iso-agglutination or iso-hemolysis occurs, may vary from a state of temporary discomfort to a grave disturbance which may be fatal. The reasons for variations in the degree is due, at least in part, to quantitative variations in the amounts of the factors involved in iso-agglutination and iso-hemolysis. The selection of donors by means of proper agglutination tests eliminates reactions of this type. Very rarely, as is referred to below, similar hemolytic reactions may occur when bloods apparently have been properly tested.

When blood is given to an individual whose serum can agglutinate the donor's red cells, the symptoms due to this incompatibility may develop after a very small amount of blood has been injected. Typically this reaction may be described as follows: The patient becomes restless, complains of pain in the back, develops an increased respiratory and pulse rate and may soon vomit and have a chill followed by a sharp rise of temperature. With hemolysis, jaundice may develop rapidly and become severe, and the urine may be scanty and filled with hemoglobin. The patient may become unconscious and appear as in shock. Death may follow rapidly or within a few days, though the severity of the reaction is usually over within twenty-four hours and the patient much more usually recovers than dies. The temperature often remains elevated for several days and the jaundice may persist for a similar length of time. The degree of anemia following severe reactions is usually more pronounced than before transfusion. Occasionally, such a reaction is followed by intense activity of the bone marrow and a surprisingly rapid improvement in the anemia occurs.

The severity of the reaction may vary greatly not only in different patients, but also in the same patient, even when the same donor is used for a subsequent transfusion. A mild reaction following a first transfusion may consist of but a very temporary rise of temperature and a chill. On the contrary, a second transfusion from the same donor may induce a severe hemolytic reaction. A presumptive explanation for this change in reaction is the development in the interim between the transfusions of an increase in strength of the agglutinins and the development of hemolysins in the patient's blood.

2. **Reactions Not Due to Recognized Incompatibility.**—These are of two types. First, those that are distinctly rare and that resemble an iso-hemolytic reaction. Second, those that are the commonest and mildest reactions that follow transfusion, and that are associated with the instability of blood when removed from the body.

(a) *Reactions That Resemble Those Due to Recognized Iso-hemolysis.*—In some diseased conditions, particularly sepsis and blood diseases, the blood sometimes seems to be altered with a production of hemolysins and agglutinins not normally present. To these abnormal hemolysins and agglutinins are attributed some of the rare reactions of a hemolytic nature which may be fatal following transfusion performed with donors selected by the usual tests. Such reactions appear to be delayed usually some hours in their onset in contrast to the classical iso-hemolytic reactions that develop at least shortly after transfusion. (See Bowcock, and Robertson and Rous.)

Sydenstricker, Mason and Rivers have observed serious hemolytic reactions following repeated transfusion in pernicious anemia, when the donors were properly chosen. The cause of these reactions is unknown. These hemolytic reactions associated with properly tested donors are not to be confused with true iso-hemolytic reactions dependent upon improper agglutination tests. Some hemolytic reactions that have been reported when the donor's and patient's blood was tested, undoubtedly have been due to improper laboratory tests. The tests were probably incorrectly read owing to the presence of weak agglutination reactions in vitro.

(b) *Reactions Associated with Instability of Blood When Removed from the Body.*—The commonest reactions seen after transfusion cannot be foretold and they are not definitely associated with agglutination or hemolysis. These reactions are of a milder nature than those previously described though they rarely may be distinctly severe. The onset of symptoms is usually about an hour after transfusion. In the majority of cases they subside within twenty-four hours. The symptoms usually begin with a sharp rise of temperature of a degree to four or five degrees, and even more. With the symptoms of fever, nausea, vomiting and diarrhea may occur. Chills may be associated with temperature rise. Urticaria, and other lesions of the erythema group, and rarely edema and purpura,

may occur. Herpetiform vesicles may develop about the mouth. The symptoms are rarely alarming and usually the reaction consists of only a simple rise in temperature.

These reactions follow the giving of blood by any method. They are apparently much more common when blood is altered by an anticoagulant than when blood is given without addition of such a substance. The frequency of such reactions varies greatly according to different observers. It seems that in round numbers outspoken definite reactions occur following transfusion of blood, as such, in about 15 per cent of the instances and with citrated blood in about 35 per cent of the instances.

Reactions of this type are generally considered as dependent upon some not clearly demonstrated alterations of blood, associated with its removal from the body. In some cases, alteration of the patient's blood seems to play a part. This is thought to be the case because these reactions appear to be commoner in patients with extensive pathology of their hematopoietic organs, such as occurs in pernicious anemia, than in those whose hematopoietic system is of a normal type, such as is found in cases with anemia due to acute blood loss.

Satterlee and Hooker, in a review of the known facts concerning such reactions, suggest three possible mechanisms by which they may be produced. One is that the trypsin-antitrypsin balance in the circulating blood of the recipient is so disturbed as to result in the immediate formation of serotoxin from cleavage products. A second theory is that the action of the protective colloids in the body cells of the recipient may be upset so that these cells are exposed to a reaction of the antigen and antibody present in the circulation of the recipient, but harmless to the protected cells. The third theory, one which is substantiated by many facts, concerns the possibility of a toxic disturbance in the circulation of the recipient by the introduction of blood which, though perfectly fluid, may be undergoing incipient coagulation changes due to the physical influences to which it is subjected in the process of transfer. The experimental work of Drinker and Brittingham and Wright and Minot, as well as the clinical results of workers experienced in the technic of transfusion, suggests that the coagulation changes may account for most of these reactions.

Novy and DeKruif attribute the toxicity of blood in the precoagulation stage to the presence of poison, anaphylatoxin, which is also present in greater or less concentration in normal serum. The mechanism of the production of this substance is the subject of an interesting theory proposed by these authors, and it may explain certain post-transfusion reactions. Novy and DeKruif believe that the matrix of the poison is always present in the circulating blood and is a substance as labile as fibrinogen, and that just as fibrinogen is changed by thrombin to fibrin, so the matrix is converted through the action of a great variety of substances into

anaphylatoxin. A foreign blood plasma could thus easily act as an accelerator of this action and suddenly convert the circulating blood into a toxic substance.

Another factor to be considered is the influence of an anticoagulant such as sodium citrate. Experience with citrated blood, as stated before, has resulted in a much larger percentage of reactions of mild type than when blood is used to which no substance has been added. Drinker and Brittingham have suggested that this may in part be due to the action on the red cells of sodium citrate which promotes hemolysis.

It is certainly true that the less blood is altered the less chance there is that these reactions will occur. Such alterations are often beyond control, for at least a small number of these reactions will develop despite scrupulous technic in transfusion. Even so, neat technic with rapid transfer of blood will permit the fewest possible reactions.

By no manner of means is it to be thought that transfusions with citrated blood should not be done, because these reactions are usually slight and rarely alarming, and fatality, if it occurs, must be very rare. However, reactions appear to be less frequent when blood without an anticoagulant is used, so that in certain instances it may be preferable not to give citrated blood.

VIII. Methods of Transfusion

Indirect methods of transfusion have entirely replaced the original direct methods. The simplicity of the indirect methods, together with the ease with which hemolysis may be avoided, has led to the general use of blood transfusion. Such methods are designed to transfer blood either as unaltered whole blood or blood mixed with an anti-coagulant, especially sodium citrate.

The chief advantage of transfusion of blood to which no substance has been added is that it produces fewer reactions, not due to recognized incompatibility, than citrated blood. In view of the reactions associated with transfusion, it is theoretically desirable to transfuse blood in its natural state as far as it lies within technical means to do so. The disadvantages encountered in the transfer of blood to which no substance has been added consist in difficulties with a more cumbersome technic for transfusion, usually requiring two or more persons, and more experience than is necessary with the citrate method. There is also a more frequent necessity for cutting down on veins when certain methods for transfusing blood without anticoagulant are employed. In the hands of experts, these difficulties are not troublesome, and in such cases transfusion of unaltered whole blood is the method of choice.

Descriptions of methods for the transfusion of blood to which no

substance has been added may be found in the papers of Kimpton and Brown, Vincent(*a*), Lindeman, and Unger(*a*)(*b*).

The reasons for the use of an anticoagulant for transfusions are simplication of technic; the necessity for haste becomes a secondary consideration and it is often more convenient since the donor and recipient need not be in the same room. One person can perform a transfusion with the citrate method, and it is usually possible to avoid exposure of veins by skin incision.

There is theoretical ground for objection to the use of sodium citrate on the grounds of toxicity, but the experience of Weil, Lewisohn(*a*)(*b*), and many others, shows that in doses up to 5 grams the drug has no demonstrable ill effects. Investigation of the effect of citrate upon the coagulation time of the blood in vivo has demonstrated that in animals the coagulation time is greatly shortened. In man, there has been observed no important change in the coagulation time after the injection of citrated blood, when the coagulation time was not abnormal. However, transfusion of citrated blood appears to be able to shorten a patient's abnormally long coagulation time in the same manner as blood to which no substance has been added. The effect of citrate upon hemolysis of red cells has been referred to.

For details of the methods for the use of citrated blood, the reader may consult articles by Robertson, Drinker and Brittingham, and Lewisohn.

Mineral Waters

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On no subject in medical literature probably has there appeared so much worthless writing as on that of mineral waters. Our own country is not guiltless but by far the largest mass of advertising under the guise of science has appeared in Europe particularly in Germany, France and Austria. While there may be virtue in many of the "drinking cures" the careful dieting and well ordered living which are a part of the "cure" are in themselves of great therapeutic value, and the ingestion of water without any mineral has very definite effects on metabolism, effects which indeed may outweigh any others attendant upon the presence of a small amount of mineral salts. While the combined action of mineral substances as they are found in natural mineral waters is undoubtedly different from that of the individual substances, it is not to be supposed that the action would be different if the natural mineral water were exactly reproduced. In considering the relation of mineral water to metabolism only such investigations as have been made with natural mineral waters themselves will in general be reviewed, since the metabolism of mineral matter is considered elsewhere. Until the laws governing mineral metabolism are more clearly understood than they are to-day the therapeutic value of mineral water administration must remain in the realm of the empirical.

A clear cut classification of mineral waters is not easily made since a water may contain several ingredients; according to their predominating characteristics, they may be divided into the following classes: saline, alkaline (including carbonated), sulphate or bitter water, sulphur, iron or chalybeate, arsenic and radioactive waters.[1]

[1] From a geochemical standpoint the fundamental character of a mineral water is best expressed in terms of the "properties of reaction" as suggested by Palmer. Primary salinity is caused by strong acid salts of the alkalies (as NaCl, K_2SO_4, etc.); secondary salinity by strong acid salts of the alkaline earths (as $CaSO_4$, $MgCl_2$, etc.); primary alkalinity is caused by weak acid salts of the alkalies (as $NaHCO_3KHS$, etc.); secondary alkalinity by weak acid salts of the alkaline earths (as $CaHCO_3)_2$, etc.) and tertiary alkalinity by colloidal oxids of iron and aluminum and free weak acids, as SiO_2 and CO_2. These "properties of reaction" can easily be calculated from a water analysis in which the values are given in terms of the ionic substance and the quality or character of the water though not its actual content of minerals, is then expressed.

Saline Waters.—The first important work on the effects of saline waters on gastric secretion was done by Dapper(*b*) on persons suffering from gastric disorders; when the usual amount of saline water was given before breakfast he was able to note normal amounts of hydrochloric acid in cases of hypoacidity due to catarrh. Hypoacidity of nervous origin was not affected, while in a number of patients hyperacidity of nervous origin was considerably reduced by the same treatment, thus indicating that the result was not merely a stimulation or inhibition of acid secretion, but a modification of the processes in the epithelium. Later work on patients (Meinel) and experiments on a dog with accessory stomach reported by Bickel(*a*), also showed that saline water given before a test meal caused a slight increase in acidity, a slightly more rapid appearance of the hydrochloric acid and emptying of the stomach.

Similar experiments on the Homburg Springs (Baumstark) (saline, CO_2) showed that these waters brought about a very noticeable increase in the amount of gastric secretion (av. 74 per cent) as compared with ordinary water, and also an increase in acid content. The opposite result appeared when milk was given with the water, from which it was concluded that the digestion period must not be identical with that in which mineral waters are ingested. The presence of CO_2 may explain the greater stimulating effect of the water alone (see below).

Sasaki, who obtained like results, claimed that the per cent of hydrochloric acid in gastric juice was not changed but that the larger amount of secretion was the fundamental thing. Casciani(*a*) and Coleschi(*a*) emphasized the fact that the hypotonic hydrochloric acid waters especially have a stimulating effect, while hypertonic waters act as depressants, isotonic having no effect. Whether the tonicity of the gastric contents as such is an important factor has been the subject of considerable experiment and discussion. The existence of a "diluting secretion" was affirmed by Strauss and Roth such that the higher the molecular concentration of a water, the longer it remains in the stomach and the greater the retardation in the appearance of hydrochloric acid (Strauss, *b*). Other investigators (Bönniger; Sommerfeld and Roeder; Otto) have not confirmed the existence of a diluting secretion and the behavior of mineral water in the stomach bears no simple relation to its molecular concentration (Tauss). However, the delay of gastric function by concentrated waters is, according to v. Noorden, a matter of therapeutic importance. Hypotonic solutions (Wiesbaden Kochbrunnen) rapidly become less so in the stomach

Since these chemical qualities are not likewise "physiological" qualities, it seemed best to retain the older and more familiar classification. As Albu and Neuberg suggest, balneotherapy may become more useful when the ionic composition of a mineral water is properly considered. While they express great hope for the future of mineral water therapy along the lines of Koeppe's investigation on the osmotic pressure and dissociation constants of mineral waters, no such development seems as yet to have taken place.

(Bickel(*a*)) and the stimulating effect of water alone (King and Hanford; Sutherland; Hawk(*e*)) first shown by Pawlow probably plays an important rôle. In this connection may also be mentioned v. Noorden's opinion that experimental results of value in therapeutics cannot be obtained in the normal organism but must be secured in one that is deranged by disease.

On pancreatic secretion saline waters have a stimulating effect (Bickel(*c*)) as shown in experiment on dogs with pancreatic fistula. The question as to the influence of these waters on the utilization of food has long been of interest and the monograph of v. Noorden summarizes his own results and those of others on persons in health and in disease. Fats especially had customarily been contra-indicated during the cures because of their supposedly defective absorption and this idea is completely refuted, for the changes in fat excretion were within normal limits, during the mineral water periods sometimes above and sometimes below the original values. This was found true even when unusual amounts of fat were ingested; no marked decrease in its assimilation occurred despite the simultaneous administration of maximum quantities of fat and mineral water together. Even small supplements of (Kissingen) bitter waters (SO_4) did not always increase the fecal content of fat and of nitrogen though their laxative action was noted.

In their long series of cases the stimulation of protein metabolism, a phrase which appears *ad nauseam* in so much of the balneological literature, was not observed. The excretion of uric acid was generally increased by drinking weak saline waters, especially in gouty patients (v. Noorden and Dapper(*a*); Leber) a statement for which v. Noorden has no explanation, but which must be accepted on the basis of the figures given; opposite findings on well persons are reported by Bain and Edgecombe and v. Noorden also observed the opposite in nephrolithiasis.

A diuretic property has also been the marvelous possession of all mineral waters. Water is the best diuretic, said Osler, and mineral waters are seldom properly compared with ordinary water nor are the relations of diet, muscular activity and external temperature and humidity ever considered. A transient diuresis (15-30 min.) is indeed often observed after drinking mineral water and the increased rapidity with which some mineral waters leave the stomach as compared with ordinary water may in part account for this; some of the salts they contain do also act as stimulants to the renal epithelium but no one has addressed himself properly to the task of determining the behavior of the kidney under the prolonged and immediate influence of mineral waters, and to the temporary and permanent effects on the body of such behavior. The ingestion of larger amounts of water (1200 c.c. in 1 hr.) with consequent enormous diuresis has very little effect on the blood according to Haldane and Priestley. Its conductance is slightly diminished whereas when salt solution is ingested its conductance is increased but hemoglobin percentage is lowered. It has

been stated that the mineral content of the blood usually increases, always within physiological limits after drinking various mineral waters, with proportionate changes in Δ (v. Szabohy; Grube), but most observations are to the effect that the molecular concentration of the blood is maintained with great tenacity (Grossmann; Strauss(*c*)) though here again the behavior of normal cases may not properly indicate that of pathological ones. The tissues rather than the blood are the regulating factors in this connection (Bogert, Underhill and Mendel).

Alkaline Waters, Including Carbonated.—Earlier work on the immediate effect of alkaline waters taken with a meal on gastric secretion was inconclusive because the variations found were within normal limits. Later work indicates that such waters taken with food have very little influence (v. Noorden and Dapper(*a*); King and Hanford). When given before meals in the usual spa fashion sodium carbonate according to some earlier investigations has very little if any effect on the secretion of hydrochloric acid (Reichmann), according to others a stimulation up to the point of neutralization and perhaps beyond (Linossier and Lemoine) to an abnormally high amount. The earlier work on Carlsbad water (alkaline-saline, containing also small amounts of Glauber's salts) which pointed to a slightly stimulating effect on hydrochloric acid secretion for the general digestion period has been supplanted by results secured on dogs with accessory stomach or human cases with esophageal fistula. According to Bickel(*b*) such water has no influence on gastric secretion, although clinically favorable results are reported both in hyperacidity and hypoacidity. However, it cannot be claimed that these effects are other than temporary and transient. Dieting, according to v. Noorden, is a much more satisfactory and efficient remedy. According to Sasaki these waters are generally slightly inhibitory, a statement with which most later investigators are in agreement (Bickel(*d*); Casciani(*b*); Heinsheimer; Pimenow; Rozenblatt).

The results obtained with alkaline-saline waters from certain Roumanian springs suggest that chlorid and bicarbonate are to an extent antagonistic in their influence on gastric secretion and that the resultant effect is dependent on the proportions present (Teohari and Babes).

Carbonated waters are generally found to be stimulating in their effect on the gastric mucosa (Penzoldt(*b*); Casciani(*a*)(*b*); Coleschi(*a*)) and also on pancreatic secretion (Becker) (perhaps as a result). The stimulating action of alkaline waters containing CO_2 is therefore to be credited to the influence of CO_2 as neutralizing the inhibitory tendency of the alkali. Gaseous CO_2 in the stomach stimulates secretion and acidity in the accessory stomach (Pincussohn) and such stimulation of alkali as has been observed is credited to CO_2 formation (Pimenow) since about the same results are obtained when using water saturated with CO_2. The effect of calcium carbonate in producing a "stormy" (Heinsheimer) increase

in secretion is likewise probably to be credited to the carbon dioxid evolved, perhaps also to calcium (Polimanti). The effect of lithium salts and water is to be explained in the same way (Mayeda).

Purely alkaline waters also depress pancreatic secretion, while carbonated waters, like the saline waters stimulate it; these also increase biliary secretion (Jappelli), all of which effects can probably be traced back to a gastric origin.

Information as to their influence on the utilization of food is scanty. Early experiments indicate little if any change in the utilization of protein and fat as a result of drinking 1 liter of alkaline water, and ethereal sulphates were also unchanged. The influence of alkalies themselves on ethereal sulphates is variable and there is need of data on the effect of mineral waters in cases of high ethereal sulphates and indican. By the ingestion of alkaline water the ammonia content of the urine is decreased, and the normally acid reaction of the urine may be changed to an alkaline reaction with sufficient alkaline water, but with wide variations in individual cases. Such results are also reported in the case of infants (Ylppö). More recently in an experiment on four men lasting 18 days the effect of an alkaline mineral water (Manitou) on digestion and utilization was determined (Mattill). This water contains a large amount of lime (secondary alkalinity), some chlorids and sulphates and a considerable amount of free carbon dioxid. During the mineral water ingestion a true alkalinity of the urine was observed together with marked reduction in urinary ammonia. There was a slight retention of nitrogen in all four subjects. Uric acid and indican excretion were very slightly reduced, the latter, however, not because of a better utilization of the food. Fecal moisture and fat in particular were somewhat increased, nitrogen only very slightly. The larger proportion of the added lime was excreted by the intestine; during the mineral water periods all subjects showed a marked retention of lime and the positive balance continued with a gradual decrease in the post-water control period. Earthy phosphates in the urine were slightly increased but total urinary phosphate was reduced, presumably through a deviation into the intestine by lime.

Alkalinization of the urine has been of interest because of the greater solvent power of such urine for uric acid. For such alkalinization the carbonates and citrates of the alkaline earths (especially calcium) offer some advantage over those of the alkalies because Ca is excreted for the most part by way of the large intestine and because, since it tends to divert phosphate from the urine to the feces (Röse) a relative as well as an absolute decrease in primary phosphates occurs (Strauss(*a*)). In his short experiment on alkaline earth waters Heim found no decrease in monosodium phosphate but the diet was not kept constant. But although alkalinized urines possess greater solubility for uric acid, the ingestion of alkaline mineral waters to provide such a condition has little or no effect

on the excretion of uric acid (Ludwig; Laqueur(*a*); Klemperer; Gilardoni; Bradenburg; Leva(*a*); Croce (*b*)); if the amount excreted is changed at all it is just as apt to be increased as decreased by alkaline waters. The same may be said of various alkalines administered as such (Herrmann; Strauss(*a*); Salkowski(*b*); Gorsky). v. Noorden remarks upon the two centuries of treatment of gout with alkalies in the absence of any findings even upon gouty patients, to justify the supposed ability of alkalies and alkaline mineral waters to remove uric acid. In nephrolithiasis on the other hand alkalies often seem to increase the uric acid output considerably.

A decreased urinary acidity is also often desirable in glycosuria and can be secured by the ingestion of large amounts of alkali (10-40 gr. $NaHCO_3$, even 100 gr. daily) amounts which are not supplied by the drinking of large amounts of mineral waters. In milder forms of such acidosis the amount of alkali in some mineral waters may be adequate to render the urine alkaline. The transitory nature of this reduction in acid is obvious as is also the fact that the reduction in acid excretion is not the real object. Any reported improvement in diabetic conditions resulting from mineral water cure can not be credited to the water but must be explained by the many other contributing factors.

The acidosis of nephritis particularly as it is related to retention of phosphates in the blood (Marriott and Howland(*a*)) requires further investigation as to the therapeutic value particularly of calcium and of the alkaline mineral waters containing it.

The fate of alkalies and their influence on the blood and tissues are questions that have not been answered for the isolated elements and their salts, much less for their wide variety of combination as they occur in mineral waters. Too little is known of the rôle of mineral substances in the processes of metabolism profitably to employ the information in a consideration of mineral waters.

Bitter Waters.—Bitter waters depress the secretion of gastric juice and may cause a secretion of water into the stomach, similar to their behavior in the intestine. In experiments on Pawlow dogs the inhibitory effect was not observed if saline and carbonated waters were added (Odaira). Acidity is said not to be markedly changed by the administration of 30 per cent sodium or magnesium sulphate solutions though pepsin is decreased (Heinsheimer). Pancreatic secretion is also interfered with (Pewsner), even by relatively small doses when food is given an hour afterward (Bickel(*c*)). These waters are laxative in their action and a less complete utilization of all the food constituents is to be expected as a result of their use. Such findings have been reported for nitrogen and fat utilization by many investigators (Leva(*a*); Vahlen; Katz(*a*); Dapper (*a*); Jacoby). In a metabolism experiment on eight persons Kolb found fecal carbohydrate also increased as well as ash. Such waters have been

found to increase urinary ethereal sulphates (Rosin) though not invariably (Porges). On the basis of urea determinations in a dog in nitrogen balance, and in patients, it was concluded that absorption of nitrogenous substances during a drinking cure was not interfered with since the urea values were not changed (Zörkendörfer). This type of water has usually been employed in obesity cures.

Sulphur Waters.—A diminished gastric acidity as the result of drinking sulphur waters has been reported from observations on a few hyperacidity cases and is recommended by Heubner(*b*) for the treatment of chronic alimentary catarrh in children. It is probable that the alkalinity of the water is the determining factor and such waters if they contain carbon dioxid may have the contrary effect (Coleschi(*b*)).

Several metabolism experiments with sulphur water are reported by Brown in which during the sulphur water periods the amount of urinary nitrogen was increased, as well as the excretion of creatinin and endogenous uric acid. The laxative action of the water caused a considerable increase in the amount of feces of which no account is taken in the nitrogen calculations. Indican was almost doubled during sulphur water ingestion. The value of sulphur water as a therapeutic agent is doubtful.

Iron Waters.—Iron waters have long been used with some success in anemia but only one investigation deals with their actual influence on metabolism. From this investigation by Vandeweyer and Wybauw on two normal persons it appears that protein and carbohydrate in the feces decreased during the iron water periods, fat on the other hand was increased. Since the nitrogen intake was not entirely uniform in all the periods, conclusions as to the effect on nitrogen metabolism are not easily drawn. In one case there was a considerable minus balance during the iron water periods as compared with the final ordinary water period; in the other case there was a plus balance, but nevertheless they conclude that during the iron water periods nitrogen catabolism is stimulated. Uric acid was relatively decreased.

The therapeutic value of iron in chlorosis is discussed elsewhere and while improvement in hyperacidity and increased hemoglobin and erythrocytes are shown to follow upon several weeks of iron water cure other factors such as rest, out of door life and proper food must be considered. The amount of iron ingested through drinking iron waters is less than is usually administered in medicinal preparations but the former are often more effective, perhaps for the reason just given, perhaps because of the manner of administration. Iron carbonate waters deteriorate when bottled and on standing due to precipitation of iron oxid.

Arsenic Waters.—Arsenic waters usually also contain iron, and for certain types of anemia it would seem that administration of iron alone is useless but that with arsenic good results are sometimes obtained. Aside from such information (Henius(*b*); Brenner) no reliable metabolism data

on arsenic waters are at hand. Uric acid elimination during the arsenic water period is said to be decreased with an increase in the after period (Croce(*b*)), but the presence of other salts is probably responsible for such results as have been noted. The excretion of arsenic in the arsenic water cures is subject to considerable individual variation (Nishi). A more rapid increase in weight in animals receiving arsenic water as compared with those receiving ordinary water has been reported for rabbits (Lardelli; Bachem) and for rats (Croce(*a*)) which is only partially explained by an effect on the appetite.

Radioactive Waters.—The literature on radioactive waters is extensive and much of its content is entirely characteristic of the bulk of mineral water literature. Radium is undoubtedly not without influence on metabolism but a great many statements about it are quite without experimental foundation. As ordinarily used in "cures" radium emanation is taken into the body by drinking radioactive water. When so taken it has no influence on gastric secretion (Olszewski). In a bath in radioactive water radium emanation enters not by the skin but through respiration (Loewenthal), but that any considerable amount gets into the blood by this means is improbable (Gudzent(*f*)) since the amount in the blood was found always to be about one-fifth of that in the expired air (Kemen). After injection into the duodenum of animals (rabbits) Strasburger(*b*) found it in three-fourth hours in the blood; after two hours only a trace was left, and the time curve of emanation content of the blood and of the expired air were the same; by divided doses the content could be maintained, but only about a third of the ingested radium emanation gets into the systemic circulation at all, and only a very small fraction is found there at any one time. Similar results were found after drinking radium emanation water. In seemingly careful experiments by Pieper the results of Strasburger were verified and it was estimated that two-thirds of the ingested radium emanation was lost by way of the lungs. A small fraction (1/4000) of the ingested radium emanation was also demonstrated in the urine from which it had disappeared after three hours (Laqueur(*b*)). In longer periods of radium emanation ingestion the amount found in the urine gradually fell (Kalmann). Radium is also excreted by the feces and in greater amounts than in the urine, and in whatever manner given it may be found in the tissues (Meyer). Thorium X seems to behave similarly and the bone marrow is said to be most rich in it after administration (Brill). Measurements of radium emanation in expired air are a good measure of the blood content (Spartz).

Radium emanation is reported as having been used successfully for the reduction of blood pressure, in the relief of anemia (Th. X), and for the cure of gout! and the literature on the latter is particularly extensive and vacuous. The supposed transformation, solution and destruction of uric acid by radium emanation (Gudzent(*a*)(*c*)(*d*); Engelmann;

Mesernitzky(*a*)(*c*)(*d*); Sarvonat) either could not be verified (Knaffl-Lenz and Wiechowski) or was found (*in vitro*) to be the result of bacteria and molds (Kerb and Lazarus) or took place just as rapidly in the body without radium emanation as with it (Höckendorff), and the cases of true gout which improved under the influence of radium emanation did not show any change in the uric acid curve (Mandel). Radium-containing waters may not even owe their value to their content of radium emanation (Lazarus(*a*)). Trustworthy information on the effect of radium or radium emanation on metabolism is meager. When given with meals certain radioactive saline waters were found to have an inhibitory effect on the action of pepsin, but only after the water had lost its radioactivity through storage (Bergell and Bickel) which the authors consider an evidence of activation of pepsin by radium emanation and a removal of the inhibitory effect of the water on gastric activity. After feeding radium bromide to dogs Berg and Welker were unable to show any change in protein metabolism; the total sulphur of the urine was increased. According to Skorczewski radium therapy causes an increased output of nitrogen and uric acid, as well as of neutral and oxidized sulphur. Using the respiration chamber Kikkoji demonstrated increased gaseous exchange and increased nitrogen and uric acid elimination which was not invariable. After intravenous injection of radium Rosenbloom found increased nitrogen elimination, but nitrogen partition showed no constant behavior. He verified the previous findings on sulphur excretion and found that the effects lasted about three days after the injection. Intravenous doses of an active deposit of radium emanation produced a decided increase in urinary nitrogen excreted by dogs (Bagg(*b*)). The destruction of cellular material as indicated by the fall in number of blood cells probably accounts for this as well as for the rise in body temperature. In a five and one-half weeks' continuous metabolism experiment on a gouty subject (Kaplan) the ingestion of radium emanation and alkaline mineral water decreased the excretion of uric acid as compared with the alkaline water alone, purin bases showed a slight absolute but a high relative increase. On the other hand, Chace and Fine found it impossible to change the uric acid concentration of the blood in gout and arthritis by emanatorium, drinking water or injections, a conclusion confirmed by others (McCrudden and Sargent(*b*)). An increased elimination of uric acid in arthritis after treating with large doses of radium emanation is considered by v. Noorden and Falta as definitely shown. This is possibly connected with cell destruction. An influence on respiratory metabolism has not been established except that after large doses a slight increase was observed (Benczur and Fuchs). A transient decrease in blood pressure has been noted (Loewy and Plesch). Despite the claims which are made for radium and radium emanation therapy in metabolic disorders (v. Noorden (*e*)) it can hardly be considered well established on an experimental basis.

Hydrotherapy . . . . . *Henry A. Mattill*

Cold Baths—Hot Baths—The Influence of Mechanical and Chemical Stimulation Accompanying Baths—Effervescent Baths—Baths and Sweat Secretion.

Hydrotherapy

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The external use of water as a therapeutic measure was first advocated in England by Sir John Floyer in 1697. A hundred years later Dr. James Currie of Liverpool, inspired by Dr. William Wright, published his reports on the effect of cold and warm water as a remedy in fever and other diseases. The works of these men bore their first fruit in Germany and Austria, where some of the claims put forth by the advocates of hydrotherapy were put to experimental test. Among the investigators Winternitz occupies the foremost place as his many monographs and his larger works testify. His efforts and those of similarly minded men that followed him have done much to illuminate the really valuable contributions of hydrotherapy shrouded as they often are under a cloud of pseudo-scientific effusions. Recent books in this country are by Baruch, Hinsdale and Kellogg. Among the recent English authors may be mentioned Fox and among the German, Matthes whose valuable chapters on baths and bathing in v. Noorden's Metabolism and Practical Medicine cites the older literature, and Schütz.

The skin is the organ through which baths produce their effects on the body. The foundation of hydrotherapy must therefore rest on the functions and activity of the skin as they may be modified by external treatment, and may in turn thereby modify the functions of the internal organs. Probably the most important function of the skin is that of regulating the body temperature, the mechanism of which is described elsewhere. By virtue of its activity in temperature regulation the skin is both a vascular organ and an organ of excretion. To the cutaneous sensations of heat and cold involved in temperature regulation must be added those of touch, pressure and pain, and the skin is thus a sense organ of first importance. The influences of hydrotherapeutic measures may therefore be sought in the effect of temperature changes and other cutaneous sensations on the processes of metabolism, including the activity of organs other than those of digestion and absorption merely, and in the effect of these stimuli on the excretory functions of the skin.

It may be recalled that the temperature of the warm-blooded animals is regulated by physical and chemical means, both mechanisms being under

the control of the autonomic nervous system. The physical regulation governs heat losses by a variable cutaneous circulation and the activity of the sweat glands. The chemical regulation controls heat production through increased muscular activity. By means of the protection of clothing, man aids these methods of regulation through surrounding himself with an atmosphere but little cooler than the body. While the internal temperature of the body is about 37.5°C. the temperature of the skin is usually only a few degrees below this, such that a bath at about 34° C. neither adds to nor subtracts from the body supply of heat. Such a bath is called an indifferent bath. This indifferent point may vary with different individuals and in different conditions and has been given variously from 34.2° to 37°.

There is fairly general agreement that exactly indifferent baths have no demonstrable influence on metabolism, whatever their duration, but while the effect of such baths or of those slightly above or below can not be measured in terms of metabolism, their importance in the treatment of many forms of insanity and in psychoses must be mentioned (Beyer). The continuous flow bath at indifferent temperature produces relief from nervous symptoms and frequently exercises a more powerful and effective sedative action than any drug. Such effects are secondary to those produced on metabolism itself but they far outweigh the latter in importance.

Cold Baths

The immediate effect of a cool or cold bath is a contraction of the cutaneous blood vessels, more or less proportional to the degree of cold, whereby loss of heat by radiation, conduction and evaporation is diminished. Depending on the extent of the cold, respiration also becomes more deep and rapid and muscular activity is excited reflexly. These responses, especially the muscular contractions known as shivering, are an attempt to produce more heat, loss of which from the body has been compensated to a slight degree only by physical regulation (Loewy). If cold application is prolonged, heat production fails to keep pace with loss, anemia gives place to hyperemia which unless it is only local (as from an ice bag) produces a rapid fall in body temperature and the circulation begins to fail. If, however, the cold is withdrawn before this time a secondary hyperemia, the "reaction" in hydrotherapy, is secured and by thus prematurely breaking off the physical regulation, the stimulus due to the temperature change is artificially enhanced. In the opinion of Matthes the stimulus due to a short exposure to cold is probably of small importance compared with the effect of the "reaction." According to Fox the whole effect of baths of every description is founded on the power of reaction possessed by the organism. The extent of the reaction is diminished

when the abstraction of heat is gradual or prolonged or when the individual is already cool or remains quiescent during and after the bath; it is increased when the application of cold is rapid and when a mechanical stimulus is added.

A transient fall in body temperature, even several degrees, may follow a cold bath and the effectiveness of a bath only slightly below body temperature in reducing fever temperature has long been known (Palmer). The contrary findings of different investigators (Liebermeister(*b*); Le Fevre(*c*); Durig and Lode) often of a single investigator on the same subject, are evidence that body temperature is not a simple resultant or that physical regulation does not behave uniformly, a possibility suggested by the ability of adaptation to repeated cold. Jürgenson found the greatest lowering of temperature by a cold bath not during but after the bath, a "primary after effect" that has been found by others (Mattill(*a*)) and may be due in part to evaporation of water retained on and in the epidermis, in part to the failure of physical regulation during the active hyperemia and its increase of heat loss. After the cooling period (5-8 hrs.) the temperature may rise higher than the corresponding daily temperature and remain there some hours as a result of the "after-effect." The duration and extent of these variations in body temperature are extremely variable (Loewy, Müller, *et al.*; Hoffman). Local applications of cold may markedly lower the temperature of the part treated as well as of the underlying tissues and organs (Riehl).

The effect of cold baths on heat production is marked and the small magnitude of body temperature changes is in fact very good evidence of the efficiency of the thermoregulating mechanism. Widely quoted figures (Matthes(*b*)) for the effect of bathing on heat production appear in Table I.

TABLE I

Effect of Bathing on Heat Production

	Temp. of Bath				
	15° C.	20° C.	25° C.	30° C.	35° C.
Heat production in calories	480	370	240	150	80
Heat—18 calories for heat loss in resp.	498	388	258	168	98
Heat—91 calories which a man of 60 kg. normally produces	407	297	167	77	7
Metabolism reduced to grams of fat.	43	31	18	8	0.7
After-effect of bath reduced to grams of fat	9	6	4	1	0.0
Total effect and after-effect reduced to grams of fat	52	37	22	9	0.7

Similar results were obtained by Ignatowski who, in a bath at 17° C. lasting 2.5 minutes found heat production 14 times normal. Of the 65

Cal. thus expended, 44 were given out during the first minute, 21 in the subsequent one and one-half minutes, and the subject was 0.3° warmer at the end. In a bath at 26.75° C. for fifteen minutes the heat loss in the three successive five-minute periods was 43, 17, and 17 calories. An abnormal loss of heat therefore takes place before physical regulation becomes entirely efficient and the cooling of the skin itself tends to reduce heat loss. This investigator also found that when his patients were really cooled down, if no "reaction" occurred heat loss after the bath continued to decrease and heat production also. With a prompt "reaction" a diminution in heat loss could not be observed.

TABLE II

Form of Bath	Duration	Temp.	Increase in Respired Air %	Increased CO_2 Output	Increased O_2 Intake	Resp. Quotient
Douch	3-5 min.	16°	54.5	149.4	110.1	0.87-1.02
Tub bath	3-5 min.	16°	22.9	64.8	46.8	0.88-1.0

Rubner's(*k*) experiments on the effects of baths and douches given in Table II show the marked effect of douches as compared with baths at the same temperature (compare mechanical stimulation below) and the respiratory quotient indicates that carbohydrates were the source of the extra energy expended. The experiments of Lusk in which men in a post-absorptive condition bathed in water at 10-16° C. are summarized in Table III. The shivering induced caused a fall in the respiratory quotient to the fasting level indicating complete exhaustion of the stores of glycogen; in one muscular individual this did not obtain. Severe shivering in one case produced a respiratory quotient of 0.67, indicating the formation of glycogen from protein, but there are no data on nitrogen elimination.

TABLE III

Form of Bath	Duration	Temp.	Increased Cal. per Kg. per Hr. %	Increased CO_2 Output %	Increased O_2 Intake %	Resp. Quotient
Subject I, Tub bath	6 min.	10°	29.	11.	34.	.99-.82
Subject I, Tub bath	8 min.	12°	33.	22.	40.	.88-.75
Subject II, Tub bath	9 min.	10°	181.	160.	188.	.95-.85
Subject II, Tub bath	10 min.	10°	116.	158.	106.	.67-.84

Observers agree that the extra energy called out by ordinary cold baths comes from non-nitrogenous material only. When body temperature falls and warm-blooded animals, obeying the laws to which cold-blooded ani-

mals are always subject, decrease their metabolism, protein disintegration rises above normal, as shown on dogs (Lepine and Flavard; Dommer) and also on men whose temperatures were reduced to 32° (Formanek(*b*)). On nitrogen distribution following cold fresh-water baths, the data of Schilling are considered reliable; he found a marked increase in ammonia excretion not associated with a simultaneous increase in nitrogen elimination. The findings of Krauss showed an increased acidity after cold baths and temporary albuminuria may often appear after prolonged cold baths (Araki(*b*); Rem-Picci). Under normal bathing conditions as employed in hydrotherapy, short cold baths cause an increase in metabolism of non-nitrogenous materials only, the energy derived therefrom being used for heat production and for the increased muscular work which this necessitates. Any energy changes due to the cooling itself are obscured by the energy expended in muscular activity and it is probable that both of these are influenced somewhat by the adaptive power of individuals to repeated heat deprivation, as well as by their physical characteristics and state of nutrition. Whether the additional heat production necessitated by cold baths takes place in the absence of muscular activity need not be discussed at this time since under ordinary conditions there is no restraint upon movement. In experiments on men it was shown that the cooling of the body in cold baths was accompanied by a rise in respiratory metabolism only where involuntary shivering occurred (Silber). It must be expected that even in the absence of such movement the additional work performed by the respiratory muscles, the heart and the vasomotor system provides some heat as a by-product.

The redistribution of blood under local or general application of cold is considerable (Hewlett, van Z. and M.) and organ activity and local metabolism are thereby modified in so far as they are dependent on blood supply. Also, since cold can penetrate more deeply than heat, it is possible to limit its effect on individual organs more accurately than is the case with heat. The general effects of cold baths on the circulatory system involve the many hydrostatic as well as reflex vascular factors affecting the bulk and the flow of the blood, and are therefore very complex. After a cold bath the pulse is slowed (Beck and Dohan), the volume pulse and minute volume are increased (Schapals), arterial blood pressure is often increased and venous pressure decreased (Winternitz(*e*); Edgecombe and Bain), the extent probably depending in part on internal compensations and antagonisms (Müller(*a*)). According to Strassburger systolic blood pressure during a cold bath may show two or three phases, a rapid rise, the more rapid as the bath is colder, a decrease (corresponding to the "reaction") and a final increase, depending on the balance between the heart action and the condition of the capillaries. After the bath there is a fall in blood pressure, usually under the original level. The transient increase in blood pressure has been given as the cause of the diuresis tem-

porarily occasioned by cold baths (Lambert), but the vasomotor changes in the skin, perhaps also in the kidney (Delezenne; Werthheimer), probably influence urine secretion somewhat. An increase in the number of erythrocytes takes place during a cold bath and is maintained for as long as two hours according to Winternitz but this is not confirmed by Tuttle. An increased elimination of urobilin after cold baths has been reported (Siccardi) and leucocytosis has also been observed (Rovighi; Thayer). The occurrence of paroxysmal hemoglobinuria after cold baths is common; a fairly complete review of this condition is given by Donath who concludes that a hemolytic property is imparted to the plasma by cold.

Cold baths usually have a refreshing effect; whether this comes as a result of modifications in the cutaneous sensations (Santlus) or in muscle sense (Vinaj) or as the result of changes in muscular efficiency (Uhlich) is uncertain.. That baths produce these changes is also questioned (Tuttle).

Hot Baths

The body possesses no chemical regulation for lessened heat production and when, in surroundings warmer than the body, the utmost heat loss by radiation and evaporation has been secured, the body temperature must rise. Rise of temperature means increased metabolism, as was first shown by Pflüger on animals and later by Winternitz and others (Ignatowski; Linser and Schmid) on man. Even moderate heating without any change in respiration causes an increase in oxygen consumption in excess of that due to fever (Winternitz(*b*)). Some of this increased heat production can be accounted for by increased work of the heart, of the muscles of respiration and of the sweat glands, but Winternitz's calculation still leaves 30-75 per cent unaccounted for, and it is probable that under these conditions warm-blooded animals, having overstepped the limits set by the heat-regulating mechanism, are subject to the effects of the general law applying to all chemical reactions.

The after-effects of a hot bath are less uniform than those of a cold bath. A continuation in the rise of body temperature after a hot or vapor bath is explained (Speck) as a natural result of the higher temperature of the skin and subcutaneous tissues as compared with that of the muscles and internal organs (Hirsch and Müller), a reversal of the ordinary condition. A compensating abnormal fall in temperature is seldom observed but in the two hours after a hot bath during which normal temperature is regained (Wick) there is a continued loss of heat in the various ways at double or three times the normal rate (Ignatowski). Winternitz(*b*) found oxygen consumption still 29 per cent above normal, 75 minutes after a hot bath. Even in hot baths of short duration without appreciable heat disturbance the volume of inspired air, the oxygen intake and the CO_2 output are increased but to a much smaller extent

than in cold baths. In both cases Rubner found the respiratory quotient rising from 0.86 to 1, as if the organism were called upon to do increased work alike by cold and hot baths. Rubner also found that an hour after a short hot bath or douche the volume of respired air and the metabolism decreased considerably, and there is thus a considerable difference in the after-effects of hot baths according to their duration. The absolute relation between the amount of heat applied and the increased heat production varies according to different investigators (Linser and Schmid; Salomon), and the differing activity of the sweat glands in physical regulation may be an adequate explanation. Marked increases in oxygen consumption, 40-111 per cent, are usually not accompanied by a proportional increase in CO_2 output, with the result that the respiratory quotient assumes low values. Similar low values are common in fever and after violent exercise, suggesting, as in Lusk's ice bath experiment, the complete exhaustion of glycogen and the breakdown of protein for its formation. An increase in protein metabolism after hot baths was long ago found in animals (Richter, Koch) and later in men (Formanek(*a*); Topp). However, Tuttle (with Folin) in careful experiments was unable to show any changes in metabolism as a result of hot baths. Since these were usually hot air baths at 190° F. or below for 5 minutes followed by indifferent and cold douches lasting one minute, or indifferent douches followed by cold douches lasting between one and two minutes, it is possible that the total heat effect was inadequate to produce changes in nitrogen metabolism. They made no determination of gaseous metabolism. An increase in protein metabolism according to Voit is not a primary result of increased body temperature but follows upon the exhaustion of readily available non-nitrogenous material since he found only a very small amount of glycogen in the liver after artificial overheating and since the administration of 30-40 gm. of sugar prevented an increased nitrogen excretion. This relation of rise in temperature to glycogen stores was not confirmed (Senator and Richter). It is probable that hyperthermia does not always cause increased nitrogen metabolism, according to Winternitz in only about one-third of the cases, and Linser and Schmid found that in fever, carbohydrate administration limited nitrogen elimination to a less extent than when the temperature was normal. According to these investigators the application of external heat even for many days does not increase nitrogen output if the body temperature remains at 39° C. or below, though when 40° C. is reached it usually does, particularly if the heating process is abrupt. They do not agree with Voit that it is a question merely of inadequate oxidizable material of a non-nitrogenous nature, and consider that in fevers the toxemia plays a part. The nitrogen loss as a result of hot baths is, according to Reilingh de Vries, only momentary since he finds that during a considerable period in which not excessively hot air baths were taken a compensatory nitrogen

retention took place, but with great individual variation, depending also on the bathing procedure and on the amount of liquid ingested. As to the nitrogen distribution in the urine, ammonia runs parallel with total nitrogen though slightly below proportionate amounts (Linser and Schmid; Schilling; Formanek(*a*)). Phosphoric acid also parallels nitrogen. There may be a very slight though not marked increase in purin bodies. The hydrogen ion concentration of the urine is increased by 15-20 minutes of heating in a sweat cabinet (Talbert(*b*)). Urinary determinations alone are not sufficient since in conditions of overheating the amount of sweat and its solid content are greatly increased.

The effects of very hot baths (105-110° F.) on pulse and blood pressure were investigated by Hill and Flack. After 15-20 minutes in such a bath body temperature rose 4-6° F., pulse increased to 160 and blood pressure fell 60, thus confirming earlier observations (Bain, Edgecombe and Frankling). They also verify previous findings as to increased respiratory frequency and volume (Edgecombe and Bain) accompanied by a notable fall in carbon dioxid tension with corresponding rise in oxygen tension. An increased systolic pressure during a hot bath was obtained by Strassburger(*a*), the hotter the bath the greater the final rise, which he considered due to increased work of the heart. The pulse volume (Schapals) and the heart volume (Beck and Dohan) are decreased. The viscosity of the blood is said to be decreased (Hess, W.) and certain of the antibodies have showed slight increase after various forms of heat treatment (Laqueur), but these changes are probably as transient as are the more readily determined variations. The non-protein nitrogen content of the blood in nephritis is not reduced by sweat baths (Austin and Miller). The oxidation of benzol to phenol in the organism is, according to Siegel, greatly accelerated by sweating processes, also by cold baths and by salt water baths more than by ordinary baths at the same temperature. The effect extended beyond the period of treatment, but there was great individual variation. It is stated that hot baths increase the secretion of bile (Kowalski) and that hot poultices or packs induce a secretion of gastric hydrochloric acid (Penzoldt(*a*)). It has long been known that the hyperemia produced by local application of heat accelerates absorption (Sässezky).

The Influence of Mechanical and Chemical Stimulation Accompanying Baths

Under this heading will be considered the effect of mechanical factors in the application of baths in ordinary water, and the mechanical and chemical stimuli arising from the presence of gases, salts and other substances in the bathing water.

The markedly increased stimulation to heat production (more than double) from a cold douche as compared with a cold tub bath at the same temperature is evident from the table given above. Winternitz(*a*) showed that the application of friction in a cold bath caused an earlier fall in temperature and a greater increase in oxygen intake and CO_2 production than a similar bath without friction. He also observed a very marked increase in heat production in a hot sand bath as compared with the results of hot air baths. Two factors, a premature breakdown of physical regulation and a direct stimulation probably come into play. Brushing the skin causes rise in temperature in man (Paalzow); so also the application of mustard paste. Mustard added to a bath at indifferent temperature increased O_2 consumption and CO_2 output by 25 per cent though without affecting the respiratory quotient. By far the greatest interest naturally attaches to sea baths and to the various other natural and artificial baths containing salts. That it is not a question of absorption through the skin is pretty well agreed upon, since the sebaceous secretions forms a barrier to water and all water soluble substances unless they act chemically on the skin. Fats and their solvents on the other hand may be imbibed by the cells or make their way through the capillary spaces and it has been reported [1] that water soluble substances may be taken up by ether-cleansed skin. Most of the investigations on sea and brine baths seem to show that their effect on energy metabolism is no different from that of baths in ordinary water at the same temperature (Jacob(*a*)(*b*); Leichtenstern) although as early as 1871 Röhrig and Zunz showed a greater gaseous exchange in rabbits in a sea salt bath than in a fresh water bath. Winternitz(*e*) concluded that such baths produce very little change in the metabolism of healthy adults, not more than 15 per cent after baths lasting one hour. The careful work of Loewy and Müller on sea air and sea baths showed an increased metabolism as evidenced by greater oxygen consumption and a decreased respiratory quotient extending beyond the duration of the bath, but there are no comparative data for fresh water bathing with similar climatic influence. The influence of salt water baths on nitrogen and inorganic especially salt metabolism has been the subject of more extended work and discussion. Some early results (Dommer) tending to show that 4 per cent NaCl baths caused a marked increase in nitrogen output (in a dog) have generally not been corroborated. The one investigator who does uphold this idea (Robin) probably had too short a preliminary period to observe nitrogen metabolism properly. Koestlin found a decrease in nitrogen excretion after warm sool baths (Stassfurt salt) while fresh water baths had no influence nor did sodium chlorid or magnesium chlorid baths, but potassium chlorid baths gave the same results as Stassfurt salts from which he concluded that potassium chlorid was the active factor. However, he did not account for fecal nitrogen or for the nitrogen given out in

[1] Kahlenberg, private communication to the author.

the sweat and his results are questioned by Bahrmann and Kochmann who conclude that even sool baths have effects no different from those of baths in ordinary water at the same temperature, nor do they trust the various reports on the usually increased chlorid excretion as a result of bathing (Keller; Robin) because of too brief preliminary periods and because the laws of sodium chlorid metabolism are not yet well enough understood. In the careful work on sea bathing above referred to an increased excretion of sodium chlorid was recorded during the bathing periods, an amount that would have required an intake of 100 c.c. of sea water, and the accidental gulping of water was avoided.

In experiments on the metabolic effects of bathing in the Great Salt Lake (20 per cent solids, mostly sodium chlorid) it appeared (Mattill(*a*) (*b*)) that the excretion of urinary nitrogen and salt increased progressively during the progress of the bathing periods. Most of the extra elimination appeared during the three hours following the bath and in amounts of from 15 to 50 per cent above the excretion during the same period on non-bathing days (Fig. 1). There was no evident compensatory decrease during the other periods of the day and the accidental swallowing of salt water was studiously avoided. The fairly uniform parallelism between nitrogen and chlorid excretion has no obvious explanation; it is similar to the findings obtained by various investigators in experiments on the influence of water ingestion. Other urinary constituents, ammonia, uric acid and creatinin were uninfluenced by the bathing. The mechanical effect of the pressure of water was much greater in this case because of the high concentration of solids, and the residual effect of the salts on the skin was correspondingly higher. This, according to Hiller may be as great as that of the bath itself. Such salts may be demonstrated spectroscopically on the skin as long as a week after a bath and various physical as well as chemical effects have been ascribed to them (Lehmann; Frankenhäuser; Schwenkenbecher). The amounts remaining after a salt bath vary with different individuals perhaps as a result of varying amount of body hair (Loewy and Müller).

The clinical investigations as to the influence of salt baths on metabolism seem to show more significant results than the purely experimental. The experiments of Heubner on two strumous children and those of Schkarin and Kufajeff on rachitic infants show that these baths have a very definite influence on the child's organism, perhaps because of the relatively greater surface area. The former investigator used gradually increasing salt concentrations and found no increase in body weight in spite of liberal feeding. Nitrogen elimination increased as the bathing period progressed with highest values in the final period leading to a negative balance in one case in which there was poorer utilization of food. In this case there was chlorid retention, in the former sodium chlorid excretion remained practically uniform. Heubner considered that metab-

olism was affected (1) by the tide of the blood between the surface and the interior of the body, and (2) by the stimulation of the peripheral vasomotor and sensory nerves. The Russian investigators in their five cases observed a considerable decrease in nitrogen retention during the bathing periods, which was not a result of poorer utilization of the food. In three cases in which a final period was also possible nitrogen retention was seen

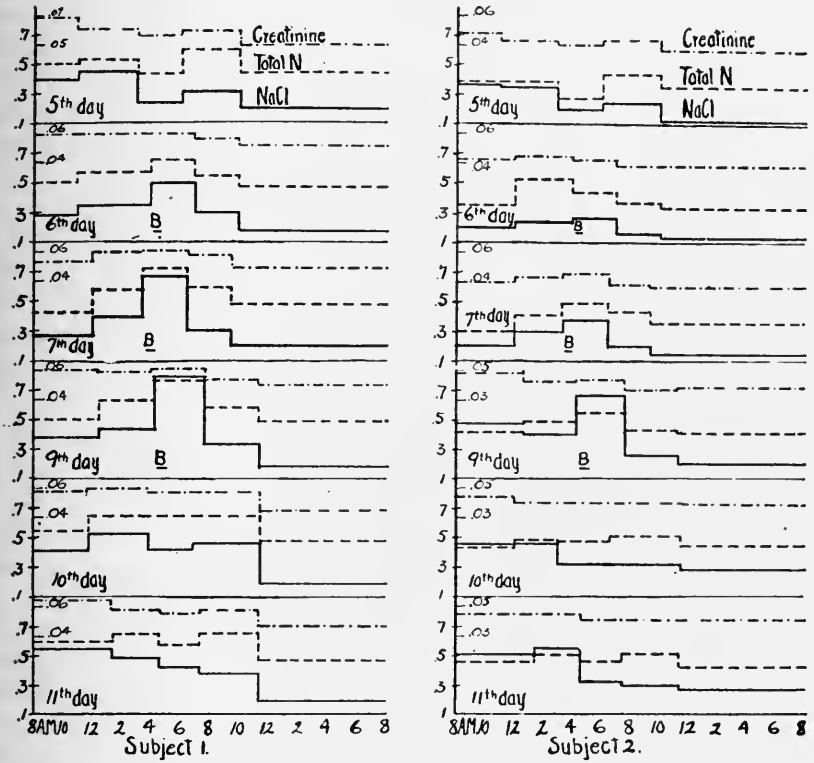

Fig. 1. Total nitrogen and sodium chlorid in tenths of grams, creatinin in hundredths of grams. *B* = Bath. (Reproduced by permission of the *American Journal of Physiology.*)

to increase toward the values found in the preliminary periods and the possibility that all children may not react to the "cure" in this way indicates that the use of sool baths in pediatrics must rest on a scientific foundation.

Blood pressure measurements made by Loewy and others in the sea-bathing experiments mentioned above showed a pronounced rise in systolic pressure, scarcely any change in the diastolic, with the result that pulse pressure reached high levels. There was usually also an increase in pulse rate. Within five minutes after the bath these phenomena had practically

disappeared. The comparative findings in a cold tub bath during which both systolic and diastolic pressures are raised and pulse is slowed show the great difference in the effects of the two kinds of baths on the circulation, and they consider that a sea bath involves, in addition to the effect of a cold bath, three factors, the salt content of the water, the mechanical effect of the waves on the skin and the muscular work involved in buffeting the waves. Similar data from fresh water seem not to be at hand. Blood pressure values following bathing in Great Salt Lake, although obtained during the bath, were normal perhaps because the factor of exercise in resistance to waves was absent.

It is a common experience that the skin feels "smoother" after a salt water bath than after a fresh water bath. This may be associated with modifications in skin sensitivity (Santlus).

Effervescent Baths

The presence of a dissolved gas in water lowers the indifferent temperature of the water; that is, the temperature at which heat is neither added to nor taken from the body. Water at 25° C. feels cool; CO_2 or O_2 at that temperature feels warmer (Senator and Frankenhäuser). In a cold effervescent bath when the body becomes covered with bubbles the points of the skin in contact with gas feel warmer than those in contact with water and the former also give off heat less rapidly since gas is a poorer conductor than water, CO_2 only one-half that of air. However, the tactile end-organs of the skin as well as the warm and cold spots are stimulated (Goldscheider) and the tendency to heat loss and to secondary heat production is greater in an effervescent bath because physical regulation is prematurely broken down by the mechanical stimulus. Hyperemia of the skin, the "reaction" appears more quickly and with less feeling of cold than in an ordinary bath at the same temperature. After due allowance has been made for these different and variable factors, it may be questioned whether an effervescent bath introduces into hydrotherapy any new features beyond the possibility of further combinations of the effects secured by ordinary procedures. The resultant temperature effect is the determining factor.

The original experiments of Winternitz showed that CO_2 baths caused an increase in the total volume of respired air and a remarkable rise in CO_2 output without corresponding increase in oxygen intake; he explained the increased CO_2 output by assuming an absorption of CO_2 by the skin. During the last two decades a very considerable body of literature has appeared on the effects of CO_2 and O_2 containing baths particularly on blood pressure (Groedel), much of it contradictory and propagandist in nature. According to Swan the influence of carbonated baths on blood

pressure is variable and any favorable results secured in cardiac cases are independent of the effect on blood pressure.

Peat and mud baths have a point of thermal indifference considerably above that of water, as high as 39° C.; in the absence of convection currents and because of the non-conducting layer next the skin the effects of heat are equalized and the skin temperature remains more constant. Possibly the chemical action of the acids and salts found in peat and mud and the physical effects of friction and pressure may affect metabolism; but there are no entirely trustworthy data as to the effects of such baths and such as are at hand (Tuszkai; Silber) do not show results that are not attributable to temperature effects on metabolism. Sulphur baths seem to have no specific influence on metabolism (Bain, Edgecombe and Frankling; Winternitz and Popischil).

Radioactive baths and springs have given the opportunity for the publication of a number of papers dealing with the supposed benefits attending their extensive use. Radium emanation does not enter the body by the skin (Nagelschmidt and Kohlrausch) and when it was added to a fresh water bath no influence on gaseous metabolism was observed (Silbergleit(*a*)).

Baths and Sweat Secretion

The influence of baths on the rate of secretion and on the composition of the sweat is of special interest because of a possible vicarious skin excretion under the influence of heat treatment, especially in diseases of the kidney. The data on the composition of human sweat are fragmentary and conflicting partly because of the wide variety of conditions under which sweat has been collected, because the composition changes with changing rate of secretion (Kittsteiner(*a*)(*b*)), varies with the different parts of the body from which it comes, and may vary with the diet (Kittsteiner(*c*); Berry). It is thus not possible to tabulate the results that have been obtained (Argutinsky; Benedict(*a*); Schwenkenbecher and Spitta; Taylor(*d*), Talbert(*a*)). The values for nitrogen elimination under different conditions vary from 0.07 to 0.75 gr. per day (or part of a day), half of which is in the form of urea (Plaggemeyer and Marshall). Salt excretion is said to vary from 0.33 gr. to 1 gram in profuse perspiration. Whether nephritics eliminate more solids in the sweat than normal persons seems undecided (Kohler; Tachau; Riggs; Loofs; Strauss (*a*)) and figures on the Δ of the blood in nephritis as influenced by sweating procedures (Bendix; Georgopulos) are not extensive enough to be convincing. Even if perspiration leads to a decrease in the urea of the urine, which it does not, always (Leube; Dapper(*a*); v. Noorden(*c*)) the amounts of nitrogenous material and salts which can be eliminated by the skin are a very small fraction of those eliminated by the kidney,

or of those present in the blood and tissues in renal disease, and in v. Noorden's opinion there is no evidence of a "vicarious" excretion on the part of the sweat glands. A reported suppression of alimentary glycosuria by rapid perspiration and appearance of sugar in the sweat (Bendix) requires confirmation. While hot baths may be of value in nephritis (Strasser and Blumenkranz) the excessive water lost in perspiration must be restored and in the light of the information on the influence of hot baths on nitrogen metabolism, the heat application should not be so powerful or rapid as to cause a rise in body temperature.

The Influence of Roentgen Rays, Radioactive Substances, Light and Electricity upon Metabolism .....

..... *Thomas Ordway, Arthur Knudson*

Roentgen Rays and Radioactive Substances—Introduction—Measurement (Standardization) of Radioactive Substances and of Roentgen Rays—Distribution and Elimination—Effect on the Blood and Blood Forming Organs—Effect on Immunity—Effect on Normal Metabolism—Effect on Metabolism in Disease—Constitutional Effects—Theories of Action—Light—Electricity.

The Influence of Roentgen Rays, Radioactive Substances, Light and Electricity upon Metabolism

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I. Roentgen Rays and Radioactive Substances

Introduction.—This discussion of the effect of Roentgen rays and radioactive substances upon metabolism will be limited almost exclusively to the more recent investigations upon man and other mammals. No attempt will be made to duplicate the comprehensive surveys of previous authors, nor can any detailed description of the physical nature of these forms of energy be considered here. In studying the effects of radiations both radium and x-rays have been used as a means of experiment and the literature of both may be considered together. As a working basis for experiment the effects of both are comparable especially in the case of the gamma rays of radium. The effect of the other rays is not however to be considered negligible but seems to differ in degree rather than in the kind of their action so that the results do not conflict with our working hypothesis.

In a survey of the subject of radiotherapy Ordway(*a*)(*b*) has briefly described the methods of use of radioactive substances and Roentgen rays for external or so-called surgical, and internal or medical conditions. He has shown that our knowledge of the former is far greater than that of the latter, which is to be advanced almost exclusively by a careful study of the effect of these physical agents upon metabolism.

Much of the earlier work has been rendered very uncertain because of the faulty physical or biological methods. It is also unfortunate that the application of the results has been in certain instances prematurely made to clinical therapeutic work on the assumption that any changes in the metabolism were necessarily beneficial.

Great caution should be used in estimating the therapeutic effect of physical agents because of the marked fluctuations which occur in the

course of chronic diseases, independent of treatment. The importance of the psychical effect of any treatment must also be considered in therapeutic work. Encouragement from the fact that something (frequently the more unusual the greater the effect) is being done is often, at least temporarily, very beneficial to patients suffering from a chronic disease. It is important to establish definitely in an objective manner how metabolism is affected by these physical agents and then to proceed very carefully to their therapeutic application.

Measurement (Standardization) of Radioactive Substances and of Roentgen Rays.—It is extremely important that detailed information of the exact technique be included in reports, so that the work may be duplicated by others. In the past the difficulty of standardizing the energy of x-rays has led to varying results and the measurement of the activity of the x-rays by their effect upon chemical pastilles or photographic films have not proven satisfactory. The development of the Coolidge tube has made it possible to secure the desired milliamperage as distinct from the voltage and the recently devised stabilizer prevents fluctuations in the current.

The relation of the methods of measurement of x-rays as expressed in erythema dose is indicated in the following table:

TABLE I

TABLE OF COMPARATIVE X-RAY DOSAGE[1]

	Designation	*Author*	*Position*
Erythema Dose	Tint B	Sabouraud	½ target skin distance
"	E 16	Kimura	½ target skin distance
"	5 H	Holzknecht	½ target skin distance
"	1-¼ H	"	Pastille on the skin
"	10 X	Kienboeck	Strip on the skin
"	4 Ha	Hampson	Pastille on the skin
"	16 Ha	"	Pastille at ½ distance
"	Coolidge tube—40 milliampere minutes at a distance of 10 inches, 60 kilovolts and *without any filtration;* 60 milliampere minutes with filtration of 1 mm. of aluminum.		

Special ionization chambers have been devised to measure the intensity of Roentgen rays. A chamber termed the ionto quantimeter for the clinical measurement of x-rays, suggested by Szillard of Paris, is described by Knox. Duane made a similar apparatus and placed it between the source of the x-rays and the object to be rayed. Glockner and Reusch

[1] Amplified after U. S. A. X-ray Manual. New York: Paul B. Hoeber, 1919.

have also described an ionization chamber for measurement of the dosage of Roentgen rays. Kronig and Friederich have made ionization chambers, the so-called iouto quantimeters, so small that they can be placed within a cavity in close proximity to the part of the body to be rayed. Such ionization chambers connected with an electroscope or an electrometer give an indication of the relative or absolute dosage of x-rays and should therefore greatly facilitate a comparison of x-rays and radioactive substances.

Estimation of the activity of radioactive substances when expressed in milligrams may be misleading unless it is based upon the activity of the gamma radiation of the radioactive element solely, as indicated by its power of ionization. This is the method adopted by the United States Bureau of Standards. Unless the standardization by weight conforms to the above there may be great variation due to the type of salt used, to the presence or absence of water of crystallization and particularly to the variable amount of impurity such as barium. The unit activity of radium salt should be expressed as above indicated in milligrams of radium element. The emanation or radioactive gas in equilibrium with one milligram of radium element has been designated one millicurie. For measuring the radioactive strength of solutions for bathing and drinking and of air for inhalation the so-called "Mache" unit is commonly used. One Mache unit is equivalent to one three-millionth part of a millicurie. Three thousand Mache units are equivalent to one-thousandth of a milligram of radium element. One-thousandth of a milligram is equivalent to one-millionth of a gram and is frequently designated as a microgram. The French formerly took the radioactivity of uranium as their standard. Uranium was considered as having a radioactivity of 1 and pure radium 2,000,000 times as great. An activity of 500,000 frequently reported in literature would represent one-fourth of pure radium and three-fourths of impurity.

In a quantitative study of the effect of radium radiations on the fertilization membranes of Nereis limbata Redfield and Bright obtained a physiological reaction to these radiations which could be measured with such precision that the thickening of the membrane served as a physiological index of the intensity of the radiation. Wood and Prime suggest for an intensity unit of radium the rays emitted by 1 milligram of radium element (1 millicurie of radium emanation) located at a point 1 centimeter distant and they designate this as 1 milligram or millicurie centimeter. Mottram and Russ consider the biological x-ray unit, which they designate by the name rad, as equal to the exposure to beta and gamma rays from 2.75 milligrams of $RaBr_2H_2O$ per square centimeter for one hour. This is just sufficient to prevent the growth of a rat sarcoma and to produce an erythema when applied to human skin.

Distribution and Elimination.—Radioactive substances differ from the x-rays from the fact that in solution in the form of a salt, or as active deposit of radium emanation, or the emanation itself in solution, they may be ingested or injected into the animal body. The emanation, the radioactive gas evolved from a solution of radium, may also be taken into the body by inhalation. A method of condensing the emanation and the deposition of the active deposit upon sodium chlorid which may be dissolved in water to make an isotonic solution has been described by Duane.

Berg and Welker found that after subcutaneous injections the radium (bromid) like barium and calcium is eliminated chiefly by the intestinal tract. Meyer after intravenous injection of solutions of radium bromide showed the presence of radium in the liver, lungs, and kidneys. The ultimate fate was not materially different if the radium was injected in any other manner, that is, subcutaneously or intraperitoneally or if a solution were taken by mouth.

Salant and Meyer conclude that the elimination of radium is chiefly by way of the liver, kidneys, and the small intestine and to a less extent through the large intestine in some herbivora. Brill and Zehner found that radium chlorid injected into dogs and rabbits was eliminated almost exclusively by the feces and there was very little in the urine. Bagg(*a*) found that following the injections of active deposit from radium emanation there is diffusion of radioactive substance throughout the animal body, resulting in pathological changes in various organs, notably the liver, lungs, kidneys, adrenals, spleen, bone marrow, brain and vascular system.

Effect on Tissues.—It is well known that radiations of Roentgen rays and radioactive substances affect different tissues to a varying degree and that the lymphatic tissue, spleen, lymph glands, bone marrow and sex glands are particularly susceptible (Heinecke and Warthin). Hauschtnig in describing the technique for radium treatment shows that the mucosa of the intestines and bladder is sensitive to one erythema dose while the muscles of the cervix uteri are resistant to forty, those of the corpus uteri to thirty, and the vaginal mucosa to five or six erythema doses. The dose which destroys carcinoma cells is practically the same as the erythema dose of the skin. Nervous tissues are very resistant to radiations.

Nakahara, and Nakahara and Murphy believe that by a carefully measured dose of x-rays (Coolidge tube, spark gap 7/8 inch, milliamperage 25, distance 8 inches, time 10 minutes) within four days there is an abnormally large number of mitotic figures found in the lymphoid tissue of the spleen and lymph glands. They believe that this indicates acceleration of the proliferative activity of this tissue by exposure to x-rays of low voltage. The great variation in the activity of lymphoid tissue naturally at different ages and also when due to intercurrent infections and

of the small number of animals in these experiments render the results uncertain.

Kimura has studied the effects of x-rays on living carcinoma and sarcoma cells in tissue cultures grown in guinea pig plasma to which was added mouse serum diluted with Ringer's solution and found that the outspreading growth was not stopped by the action of the x-rays with a dosage of E 4 to E 12. The mitotic figures were limited to a minimum after an exposure to a dosage of E 8 and after an exposure to E 12 the mitoses disappeared entirely and the tissue so treated produced no tumors when inoculated into mice. The growing power of the sarcoma after exposure to a dose of E 4 was apparently somewhat stimulated and the carcinoma was not appreciably influenced. The process of oxidation of the tissues in both the sarcoma and carcinoma cultures was stimulated by x-ray action of the dosage of E 4 and retarded by exposures to E 12.

The histological changes in tissue, induced by exposure to radiations of x-rays and radium, have been described in detail by many investigators. They consist of a necrobiosis of the cells, a chronic inflammatory reaction, followed by fibrosis. The changes depend on the intensity of the radiation and the type of tissue radiated.

Effect on the Blood and Blood Forming Organs.—The chemical effect of radiations of radium and x-ray upon the blood will be referred to later. Gudzent(*g*) has summarized the work prior to 1913. It may be briefly stated that the lymphocytes are apparently stimulated to both relative and absolute increase by small doses and reduced in number by large doses of x-rays; and that the spleen and lymph glands undergo profound change by destruction of the cellular elements as the result of exposure to x-rays and radium. Gudzent and Halberstaedter found in the blood of radium workers striking relative increase in lymphocytes (36 to 63 per cent), in an average of ten cases 40.4 per cent and a relative and absolute decrease in neutrophils, the average number being 50.3 per cent. There was little change in the red blood corpuscles, slight diminution in the white cells, the hemoglobin was lowered in only two cases, 70 and 71 per cent respectively. Ordway(*c*) found a similar though somewhat less marked change in a series of clinical workers who showed local occupational injuries due to the handling of radium.

Millet and Mueller in a study of the blood of ten patients with squamous cell carcinoma of the cervix uteri and the vagina, for the immediate and remote effects of radium and x-rays, found an immediate drop in the total white count reaching a maximum in one-half to six hours after application, and a return to normal within twelve to twenty-four hours. Occasionally there was a secondary rise in from 12 hours to 3 days. The polymorphonuclear count followed the total white count. The total lymphocytes tended to follow the white count but were not constant. There was a tendency for the relative lymphocyte count to drop and the poly-

morphonuclear to rise during treatment but this tendency was reversed immediately following the removal of the radiations. The remote effects consisted of a fall in the lymphocyte count for two to four weeks after treatment, sometimes lasting until the end of the second month. The fall in the polymorphonuclears was usually less than the lymphocytes, the latter after from three to nineteen weeks rose to the normal level. When the patient's resistance weakened they found an increase in the polymorphonuclear leucocytes and decrease in the lymphocytes but without leucocytosis due chiefly to an absolute increase in the polymorphonuclear leucocytes and usually a decrease in the lymphocytes. Such changes in the blood, however, are subject to considerable fluctuations owing to secondary infections. This is not only true in human beings but particularly in the experimental study of radiation effects in the blood of animals.

Woglam and Itami have shown that it is not easy to establish a normal standard for certain laboratory animals, notably mice, that there is great variation in the activity of the hematopoietic tissues in apparently healthy individuals. The age as well as intercurrent infections are factors which must be taken into consideration.

Aubertin and Beaujard studying the action of x-rays on the blood and bone marrow show that the marrow is much less sensitive to raying than the lymphoid tissue. They believe that leukopenia may be produced by the x-ray, either by the direct action of the rays upon the leucocytes in the circulation or by its action on hematopoietic tissue which prevents normal regeneration of white blood cells. Brill and Zehner injected a soluble salt of radium ($RaCl_2$) subcutaneously into dogs and rabbits in doses of 0.0025 and 0.093 mgm. and found that almost immediately the number of red cells per cu. mm. was greatly increased. On the day following there was another marked increase. This polycythemia persisted for a week and for several weeks the number of red blood cells was considerably above normal; the hemoglobin did not rise so markedly. The leucocytes increased rapidly after small injections and in certain instances rose to 200 per cent above the normal. The larger injections on the other hand inhibited leucocyte production.

Effect on Immunity.—X-rays and radioactive substances have such a pronounced effect on the blood and blood forming organs, the bone marrow, spleen, and lymphoid tissue generally that it is not surprising that variations in immunity and susceptibility are produced by exposure to radiations. Hektoen (*a*) (*b*) found that long exposure to x-ray at the time the antigens were injected into white rats markedly reduced the production of hemolytic antibodies. He assumed that this was due to the destructive effect on the lymphatic tissues, spleen and bone marrow. In some further experiments he exposed dogs to x-rays for ten minutes, followed the next day by a two and a half minute exposure (approximately 37½ Kienboeck units); they showed slight apparent disturbance of general health and no

great change in the leucocytes in the peripheral blood but there was a marked reduction in the production of antibodies hemolytic for red blood corpuscles of the rabbit.

Morton found that exposure of guinea pigs to x-rays rendered these animals more susceptible to experimental tuberculosis and suggested such preliminary radiation for the routine diagnosis by the guinea pig method. Kessel and Sittenfield, however, believe that after a certain stage radiation tends to prolong the life of a tuberculous guinea pig and to promote healing. Kellert finds that in routine work preliminary radiation does not hasten the diagnosis by rendering guinea pigs more susceptible to tuberculosis but that the increased susceptibility of such animals to secondary invaders and contaminating organisms interferes with the routine work. Corper and Chovey, by subjecting mice to a single non-lethal dose of x-rays or to a single non-fatal injection of thorium-x, subsequently found that these animals showed an increased susceptibility when inoculated with pneumococci (four types) and hemolytic streptococci (human and bovine).

Russ, Chambers, Scott and Mottram in experimental studies with small doses of x-rays, following the work of Murphy and Morton(*a*), on the blood of rats in its relation to rat susceptibility in Jensen rat sarcoma find that the natural immunity which these animals have towards inoculation of spontaneous tumors can be broken down by an x-ray exposure sufficient to cause the disappearance of the lymphocytes. Prime on the other hand did not succeed in rendering rats naturally immune to the Flexner-Jobling rat carcinoma, more susceptible by reducing the lymphocytes as advocated by Murphy. Murphy and Taylor have shown that the acquired immunity resulting from the inoculation of blood or other cells into normal animals can be similarly destroyed. The acquired immunity found in animals in which tumors have disappeared, according to Mottram and Russ, can be broken down only so long as lymphoid cells are reduced in number. Tumor cells from a foreign species which on inoculation will grow only with great rarity multiply rapidly in an x-rayed animal until such a time as the depleted lymphoid tissues are well advanced in regeneration (Murphy).

On the other hand Russ, Chambers, Scott and Mottram, and Murphy and Morton(*a*) have shown that an immune condition can be produced instead of destroyed by suitable doses of x-ray. After the removal of tumors from mice by operation Murphy and Morton(*b*) gave small dose of x-rays and found that grafts of the same tumors when inoculated did not grow in twenty-six out of fifty-two mice and that there was no recurrence at the site of operation in forty-one animals. In twenty-nine control mice who were not given small doses of x-rays the grafts grew in twenty-eight and there was local recurrence in fourteen.

From the above it appears that the x-rays have two actions aside from the direct effect upon the tumor. First a large dose destroying the immune condition will favor the growth of tumor, a small dose producing the immune condition helps to inhibit the growth of tumor.

Such studies indicate that in treating growths by radium or x-ray a treatment directed solely toward the primary growth may favor metastasis by lowering the natural powers of resistance of the patient, especially if comparatively large doses are repeated at too frequent intervals. Murphy believes that great caution should be used about destroying the lymphocytes which seem to play the defensive rôle in malignant growths.

Up to the present time the x-ray has only increased the resistance to inoculated cancer. Yet there is a distinct analogy between such and metastatic deposits of a spontaneous growth. Hence it is suggested by Murphy that repeated small doses of x-rays at intervals might similarly increase resistance against the development of secondary, metastatic growths.

Rohdenburg and Bullock by heat and exposure to radium have increased the susceptibility in mice and rats to the immunizing action of homologous living cells and the additional immunity thus obtained may be one hundred per cent over the usual figure. The growth energy of transplanted tumors also can be depressed by radium (Wedd and Russ). This retardation of growth energy persists only a few generations of transplants (Wood and Prime).

Believing that there might be a relation between the number of lymphocytes in the disease poliomyelitis and the susceptibility of monkeys to experimental poliomyelitis Amoss, Taylor and Witherbee reduced the circulating lymphocytes in such animals by properly controlled doses of x-rays such as were used by Taylor, Witherbee and Murphy. Six Holzknecht units of unfiltered x-rays was given at each dose on the dorsal and ventral surface of the animal. Spark gap was three inches, milliamperage ten, distance twelve inches (Coolidge tube), time four minutes. The animals were treated every day or every other day until the total lymphocytes per c.mm. were about 1000 to 2000. Animals thus exposed to x-rays were susceptible to three-fourths of a dose which was not infective for non-rayed controls. This suggests a relation between the lymphocytes and one factor of resistance in poliomyelitis. They were not able to reduce the immunity by exposure to x-rays in a monkey immune from a previous attack of poliomyelitis.

Effect on Enzymes.—Richards(*b*) believes that the biological reactions resulting from exposure to radiations are due in large part to the effect upon the body ferments. Richter and Gerhartz in studying the action of x-rays upon rennin, yeast, pepsin, pancreatin and papain concluded there was no effect on these ferments. Richards(*a*), however, believes that the experiments of these authors do show slight changes which may be at-

tributable to the effect of x-rays. He concludes from his experiments on the digestion of egg albumin by pepsin and of starch by diastase that a short radiation by x-rays has the effect of accelerating enzyme activity while a longer radiation inhibits it, and that between these two intervals there is a non-effective point. The experiments of Richards show that the effects are slight but definite.

Radium rays, which are in general comparable with x-rays in their action, have been thought to be the cause of quite marked changes in the course of enzymatic action. Neuberg(*b*) found an acceleration of the autolytic processes under the action of radium emanation. Packard considers that radium radiations, by activating autolytic enzymes, act indirectly upon the chromatin and protoplasm and thus bring about the degeneration of the complex proteins and probably affect other protoplasmic substances in the same manner. Influence of radium emanation upon autolysis of normal and pathological tissues has been studied by Lowenthal and Edelstein. They found that the rate of increase in autolysis varied with the character of the material allowed to autolyze, but the greatest accelerating influence was found in the case of human carcinoma.

Henri and Mayer in studying the action of radium on ferments found that invertin, emulsin and trypsin exposed to radiations decreased and finally lost their activity. Bergdell and Bichel observed that the activity of pepsin is enhanced by the influence of radium rays. Schmidt-Nielson showed that radium preparation of 1,800,000 activity has slight inhibiting action upon rennin. Wilcock has reported that radium rays are injurious to digestive ferments such as pepsin, trypsin, and ptyalin. According to Lowenthal and Wolgemuth radium emanation is capable of accelerating the activity of the diastatic enzyme of the blood, liver, saliva, or pancreas, that there may be a slight retardation which is replaced by acceleration if the experiment is sufficiently prolonged. Brown found that the very radioactive radium D, radium E, and radium F have a marked inhibitory action upon pepsin and pancreatic diastase; but no effect upon the autolytic enzyme of the dog's liver. Marshall and Rowntree's(*a*) investigation showed that the radium emanation has no accelerating influence upon the lipase of the pig's liver or castor oil bean, while inhibition of the enzymatic activity is suggested. Schulz(*b*) observed that radium emanation has a certain amount of accelerating action upon the uric-acid forming enzymes of the spleen.

From the fact that alterations in permeability may cause cell division and such metabolic changes as increased elimination of carbon dioxid, of catalase, and an increase of oxygen absorption and various other physiological reactions in the cell Richards(*c*) performed experiments on x-radiation as a cause of permeability changes but was unable to find any evidence that alterations in cell metabolism are due to permeability changes. Min-

ami has shown that thorium-x emanation accelerates or retards peptic, tryptic and diastatic digestion. The duration of such action depends in part on the time the radiations act. He believes that possibly the autolytic ferments are influenced by the alpha rays.

From the foregoing it will be seen that radiation affects enzymes definitely, but the effects are variable, probably depending upon the duration or the amount of radiation.

Funk(*e*) investigated the influence of radium emanation on the yeast vitamins and reported that radium emanation has no destructive action on beri-beri vitamin or on the growth-promoting factors in yeast. Suguira and Benedict, however, subjected portions of yeast to the rays of radium and tested this for their growth-promoting powers upon young white rats as compared with the same yeast not treated with radium. They observed that the growth-promoting factors in yeast may be partially inactivated by means of exposure and believe that this may account for some of its effects on tumors.

Effect on Normal Metabolism.—Most of the contributions dealing with metabolism studies under the influence of radioactive substances and x-rays have been concerned with abnormal human beings, but some work has been done upon normal animals and human beings. Quadrome studied the influence of x-rays on one guinea pig and six rabbits and although his results were not uniform he got in most cases a slight increase in the urine of the total phosphates (P_2O_5). Baermann and Linser obtained an increased nitrogen excretion immediately after raying their patients; this increase lasted two or three days and on the third or fourth day the nitrogen excretion usually returned to normal. In a man, normal except for chronic eczema, Bloch observed after repeated raying a small increase of basic nitrogen output in urine also an increase of phosphates. The metabolism of one dog rayed with large doses of roentgen rays was studied by Benjamin and V. Reuss. An immediate increase in nitrogen elimination was observed after the first exposure and rapidly returned to normal. In a second exposure to the rays the increased elimination lasted several days. The basic nitrogen (product formed by precipitation with phosphotungstic acid), non-basic nitrogen, ammonia and urea, which were determined on the urine specimens along with the total nitrogen, all showed an increase. The basic nitrogen increased proportionately more than the others. The phosphate output of the urine also increased transiently. In the first exposure it rose to 33 per cent above normal and the second to over 100 per cent. During the high phosphate output in the urine a transient appearance of cholin in the blood was demonstrated, which the authors attributed to the breaking up of lecithin and substances derived therefrom. Metabolism observations reported by Lommel on three young dogs showed similar results; that is, increased nitrogen and phosphate elimination. Linser and Sick, in studying the effect of x-rays on

several individuals with various skin diseases, noted in all an increase in the urinary nitrogen. The uric acid output was tripled in some cases and the purin bases also increased. Similar results were observed in one experiment on a normal dog.

The effect of radium salts upon the metabolism of dogs has been studied by Berg and Welker. The doses employed were very small and they concluded that the ingestion of radium per os was without any special influence on metabolism. In one experiment a stimulation of the catabolic processes as indicated by slightly increased output of nitrogen in the urine was noted, but in another experiment the catabolic processes were inhibited to about the same degree. An increased volume of urine was also noted. In order to determine the effect of the active rays upon the general metabolism of the dog Theis and Bagg used a solution of sodium chlorid which contained active deposit from radium emanation. The dogs were given doses of two to six millicuries per kilogram. One dog was a Dalmatian in which variety uric acid is excreted in the urine. The total nitrogen in the urine always increased reaching a maximum of ten to twenty-five per cent on the second day after injection. Urea nitrogen paralleled the total nitrogen, but the ammonia nitrogen increased in greater proportion than the total nitrogen indicating a possibility of acidosis. Uric acid in the Dalmatian dog inereased both absolutely (15 to 50 per cent) and relatively to the total nitrogen. This may have been due to the destruction of the white cells for the phosphate excretion was also increased. Creatinin in one experiment was increased but not proportionately to the total nitrogen. Jastrowitz has recently reported that injection of thorium into dogs has a tendency to increase excretion of uric acid above normal.

After deep massive doses of hard Roentgen rays Hall and Whipple noted marked metabolic changes in experiments on dogs. The nitrogen excretion of the urine increased immediately following exposure to rays and remained high until death. There was often an increase of fifty to one hundred per cent above normal. A marked increase (twice normal) of the non-protein nitrogen of the blood was commonly observed on the day before death and often more than three times normal on the day of death. The authors do not believe that the heaping up of nitrogenous split products can be explained alone on an increased breakdown of body protein but that there may be faulty elimination. They could observe, however, no evidence of any nephritis from a study of the urine nor by anatomical changes.

Denis and Martin in studying the relative toxic effects produced by regional radiation found that exposure with massive doses of Roentgen rays over the intestines of a rabbit gave evidence of the presence of an acidosis. This was shown by a fall in the alkaline reserve and a rise in fat and inorganic phosphates of the blood of most of the rabbits which

received the heavy exposure over the intestine. In some of the rabbits a slight increase in non-protein nitrogen was also noted.

A number of investigations on the influence of radioactive substances and x-rays on uric acid and purin base metabolism have led to the general belief that these agents lead to an increased elimination of uric acid and purin bases, endogenous as well as exogenous. Gudzent and Lowenthal believe that radium emanation has a very pronounced effect on purin metabolism and is due to the activation of those enzymes responsible for the building up or cleavage of uric acid. Purin metabolism is altered according to whether synthesizing or cleavage enzyme action predominates. Wilke and Krieg report increases of uric acid excretion with ingestion of radioactive water. Kikkoji obtained a similar result with water impregnated with radium emanation and in one of his cases observed an increase of ninety-five per cent. Kaplan reports that ingestion of alkaline radium water increases the excretion of uric acid and purin bases. Abl also observed increased elimination of endogenous uric acid by use of thorium-x.

The mechanism of these effects is not established. Gudzent(*a*)(*d*) claims to have induced a complete and lasting disappearance of blood uric acid by inhalation of air containing two or four Mache units of emanation per liter. In apparent confirmation of this fact he noted in vitro experiments an increase in the solubility and gradual decomposition of sodium urate by radium D, which is relatively very inactive and is a further decomposition product of radium emanation. Falta and Zehner claim that thorium-x also increases the solubility of urates and destroys uric acid. Mesernitzky(*b*)(*e*) reported that radium emanation can destroy trioxypurin (uric acid) very well but that it had slight effect on dioxypurin (xanthin) and no effect on oxypurin (hypoxanthin). He also claims that uric acid in the blood is decreased under the influence of radium emanation and that there is an increased excretion of uric acid in the urine. Other observers have been unable to confirm these results. Kerb and Lazarus were unable to detect any influence of radium emanation upon sodium urate. Using radiation from radium emanation in very large amounts Knafft-Lenz and Weichowski likewise failed to note any increase in the solubility or decomposition of sodium urate. Kerb and Lazarus were of the opinion that the increase in solubility and decomposition of sodium urate noted by Gudzent is to be attributed to bacterial contamination or accidental introduction of small amounts of alkali, either of which conditions could cause decomposition of the urate.

Schultz could detect no change in the activity of the uricolytic enzyme of the liver and kidney under the influence of radium emanation but did observe a ten to twenty per cent increase in the formation of uric acid in autolyzing spleen under these conditions. This latter observation and that of Kehrer (which bespeaks a mobilization of uric acid in the

body attributable to emanation) would lead one to expect, if any change at all, rather an increased concentration of uric acid in the blood than a decrease, much less complete disappearance as Gudzent would have us believe.

Investigations by Fine and Chace with inhalation of radium emanation (containing as high as one hundred Mache units per liter) over long periods, radium emanation in drinking water, and injection of fifty micrograms of soluble radium bromid in no case had any influence whatever upon the concentration of uric acid in the blood. Likewise they could observe no increase in the excretion of uric acid in the urine.

Very few observations have been made on the effect of radiation on the basal metabolism in normal animals and human beings. Silbergleit(*b*) studied the influence of baths containing radium emanation on the gaseous exchange of normal men, but his results were negative. Kikkoji found a distinct increase in the basal metabolism of normal men who received during the experimental period three doses of 330 Mache units per os. The respiratory quotient was also sometimes increased. Bernstein determined the basal metabolism of several persons before and after a two-hour interval in an emanatorium containing from 220 to 440 Mache units per liter of air. One of these was carried out on a normal individual and showed an increase of about six per cent. A slight increase of the respiratory quotient was likewise noted. The respiratory quotient remained practically unaffected according to Benczur and Fuchs(*a*) with ingestion of radium emanation water containing 300,000 to 400,000 Mache units. With radium alkaline waters Stachelin and Maase found the gaseous exchange considerably decreased. This decrease refers only to values following the taking of food and not to fasting values.

The carbohydrate metabolism is apparently increased according to the observations of Kikkoji and Bernstein who found in their basal metabolism studies an increase in the respiratory quotient in most cases. Lipine(*c*) found that exposure of dogs to x-rays for one hour is followed by an increased glucolysis which is more marked if impacted with eosin before radiation.

That radioactive substances and x-rays have an effect upon normal metabolism is well established by the results of investigations reported above. According to Musser and Edsall the effect of x-rays upon metabolism is unqualled by any other therapeutic agent and we might apply that statement equally to radium. The changes produced by these agents is manifested by an excessive elimination of the products of protein destruction indicated by the increased elimination of total nitrogen, uric acid, purin bases and phosphates, and the accumulation in some cases of non-protein nitrogen in the blood. That these agents have an effect upon carbohydrate metabolism and fat metabolism is not so well established by the meager results so far reported.

The cause of these effects on metabolism is at present difficult of explanation. One may ascribe the effects of x-rays either to a stimulating effect upon autolytic enzymes or as Neuberg(*a*) does to an inhibitory action of x-rays and radium rays upon the other intracellular enzymes without corresponding deleterious effect upon the autolytic enzymes present. This hypothesis agrees with the facts at hand but more details concerning the effects of these rays upon various enzymes are needed.

Effect on Metabolism in Disease.—The metabolic changes produced by x-rays and radioactive substances in various diseases have been studied quite extensively. The protein destruction by these agents arising partly from the lymphatic structures has led to their study particularly in connection with the treatment of leukemia. Following the therapeutic use of x-ray and radium in leukemia there has been observed a marked effect on metabolism.

Lossen and Morawitz in a case of myeloid leukemia treated by x-rays found that the volume of urine was decreased, that total nitrogen, uric acid and phosphorus excretion lowered. Heile found an increase in both uric acid and purin bases in three cases. Koniger in myeloid leukemia found that under influence of Roentgen rays the uric-acid excretion increases parallel with the diminution in size of the spleen and the breaking up of the leucocytes and that the uric-acid excretion is a positive measure of cell breakage, but not an index to the extent of the cell destruction. Ammonia and phosphates were also increased at times, generally parallel with the nitrogen increase and also with the betterment in the leukemic symptoms. No increase in the total nitrogen or uric acid could be found, however, by Cavina in a case of lymphatic leukemia treated with Roentgen rays.

In this connection the observations of Musser and Edsall are of interest. In those cases in which the roentgen ray caused a reduction in number of white cells and there was clinical improvement, there was a definite increase in uric acid and purin base output, a marked loss of nitrogen and an increased elimination of phosphates. In a case in which x-rays had no beneficial effect clinically, there was likewise no effect or very little on the nitrogenous metabolism.

Murphy, Means and Aub studied the basal metabolism of a man with chronic lymphatic leukemia. Observations were made before and after exposure to x-ray and also after exposure to radium. When first observed the metabolism was 44 per cent above the average normal, falling a little with rest in bed. Intensive treatment with x-rays caused a drop in the leucocyte count but did not appreciably affect the level of the metabolism. Water elimination through the skin and respiratory passages was unusually high. Direct and indirect calorimetry gave total results which were almost identical and no abnormal respiratory quotients were found. After treatment with radium a further very marked fall occurred in the leuco-

cyte count, at the same time there was a slight fall in the basal metabolism.

Radium has been found to have a similar effect upon the nitrogenous metabolism in leukemia as do x-rays. Knudson and Erdos in a case of myelogenous leukemia treated by surface application of radium observed in each of the three series of treatments marked changes in metabolism. The total nitrogen, urea, ammonia and phosphates are immediately increased and reach a maximum in about seven days after each application. The uric acid excretion also increased some the first seven days and then remained at about the same level throughout the observations. An examination of the uric acid in the blood at relatively long intervals during the treatment showed little change. In another case of myelogenous leukemia, Ordway, Tait and Knudson obtained results in conformity with the case described above. An examination of the blood for creatinin and non-protein nitrogen before, during and immediately following radium treatment shows that there is apparently no change during the radiation.

Martin, Denis and Aldrich have studied the chemical changes in the blood following Roentgen ray treatment in leukemia. In the more severe cases they found the non-protein nitrogen was high and after treatment a gradual but steady fall was noted. The creatinin was not affected. The uric acid content was much increased but a large diminution in the number of white cells which occurred as a result of treatment caused no appreciable decrease in this constituent.

The iron metabolism in myelogenous leukemia before and after exposure to x-rays has been studied by Bayer(*b*). He found that isolated exposure of spleen to x-rays causes an absolute increase in iron excretion in the feces greater than in the isolated exposure of the long bones. The iron excretion in pathological conditions of the spleen is greater after exposure to x-rays than in the normal.

The chemical changes observed in the treatment of leukemia with x-rays and radium apparently depend upon the excessive quantity of leucocytes and lymphoid tissue, which undergo processes of disintegration during treatment, with the result that products of nucleoprotein destruction (total nitrogen, uric acid, purine bases, and phosphates) appear in the urine in increased quantities.

The use of radium in the treatment of gout directed early the attention of investigators to the influence of radium on uric-acid metabolism. As a result of the investigations in His' clinic it was affirmed that uric acid occurs in the blood in gout in a specially insoluble modification and that under the influence of radium the insoluble pathological form of uric acid becomes changed to a more soluble physiological form which is easily destroyed and excreted; the net result being a rapid solution of the gout tophi, an increased elimination of uric acid in the urine and a disappearance from the blood (Gudzent and Lowenthal, Gudzent(*a*)(*b*)(*d*)).

The experiments on which these investigators based their theory of gout and action of radium were at first apparently confirmed. Mesernitsky and Kemen, Kikkoji; Von Noorden and Falta, and Skorczewski and Sohn report increased excretion of uric acid in cases of gout under the influence of radium emanation. Plesch and Karczag observed a similar effect with thorium-x.

With reliable methods and carefully controlled observations Chace and Fine could not confirm these observations. Inhalations of radium emanation (containing as high as 100 Mache units per liter) and injection of fifty micrograms of radium bromid in no case had any influence upon uric acid concentration in the blood of patients with gout. McCrudden and Sargent(*b*) likewise could observe no effect on the concentration of uric acid in the blood of a patient with gout receiving water impregnated with radium emanation. The patient received daily 20,000 Mache units. No effect could be found on the rate of uric acid and total nitrogen excretion but they did observe a slight increase in the creatinin excretion which persisted for a few days after discontinuing the radium treatment.

Chace and Fine and McCrudden and Sargent(*b*) have also studied the effect of radium emanation on cases of chronic arthritis. They could observe no effect on the concentration of uric acid in the blood or the rate of its excretion in the urine. McCrudden did observe, however, a slight increase of creatinin excretion. In a case of rheumatoid arthritis treated by intravenous injection of fifty micrograms of radium salts Rosenbloom(*b*) noted an increased nitrogen excretion and a marked increase in the amount of total sulphur and neutral sulphur in the urine. The increase of nitrogen and sulphur lasted for about three days following the injection.

The metabolism of cases of pernicious anemia, rheumatoid arthritis, and unresolved pneumonia treated by x-ray have been reported by Edsall and Pemberton. In the cases of pernicious anemia and rheumatoid arthritis x-ray exposure produced a toxic reaction. The chief point of interest in these two cases is the remarkable drop in excretion of nitrogen, phosphates and uric acid that followed the exposure. The drop was followed subsequently by an equally striking rise in excretion to a point much beyond that at which it had previously been. In the first case the drop occurred directly after exposure and in the second it was postponed two days but occurred as in the first case when the man had become seriously ill. In the cases of unresolved pneumonia the effects were striking. There was an immediate marked increase in the nitrogen and chlorid excretion. The phosphates were increased somewhat less and uric acid was little affected. This effect upon metabolism was coincident with a rapid improvement. The only apparent explanation the authors give to these results is that in those cases, such as unresolved pneumonia and leukemia, which responded favorably to x-ray treatment an increased tissue destruction occurs directly after exposure resulting in an increased excretion of

the products of metabolism. The cases without an immediate increase in the nitrogen excretion were unfavorably influenced by x-ray application. It seems to the authors that the organism in these two cases was overwhelmed by the enormous amount of the products of tissue destruction, resulting in a retention of decomposed tissue products. After a time the organism reacted somewhat and a complete distintegration could be accomplished and the products were excreted.

Ordway, Tait and Knudson have studied the influence upon metabolism of surface application of radium emanation upon a case of sarcoma and of carcinoma respectively. In the former they observed increases in the volume of urine, in total acidity, ammonia, total nitrogen, urea, and uric acid. Creatinin and phosphates were considerably increased. In the case with carcinoma there was no increase of the nitrogenous fractions or phosphates of the urine. The changes in the nitrogen metabolism depend apparently upon the amount and nature of tissue autolysis. In the case of sarcoma there was a definite softening and fluctuation of the growth while in the case of carcinoma of the breast the lesion consisted of hard brawny fibrous tissue in which one would expect little or no autolysis.

Ludin has observed that radium reduces the high cholesterol values observed in the blood of carcinoma patients and emphasizes the fact that this may play an important part in the beneficial effect of radium therapy. De Niord, Schreiner, and De Niord have studied the influence of Roentgen rays on the blood of cancer patients in order to note whether radiation produces any appreciable change in their blood chemistry. Blood specimens were taken before exposure to x-rays, one half hour and twenty-four after exposure. Radiation had no effect upon the sodium chlorid content nor upon the percentage of corpuscles and plasma. The changes in the urea nitrogen, creatinin, uric acid, sugar and diastatic activity are inconsistent, which makes it difficult to draw any conclusions. In a number of the cases these constituents were found to be increased and in an equal number they were found to be decreased or to have no effect. The cholesterol, fatty acids and total fats were found to be generally increased in the cases of malignancy. After exposure to x-rays the total fatty acids were found to be reduced in 72 per cent of the cases and the total fat was reduced in 83 per cent. The cholesterol content in 61 per cent of the cases was higher and in 31 per cent was lower after exposure. The increase in cholesterol was not proportional to the time of exposure or the type of tumor.

Rudinger studied the influence of Roentgen rays on protein metabolism in Basedow's disease. He found exposure to the rays induced a retention of nitrogen as indicated by a gradual fall of elimination. No relation could be found between the phosphorus and nitrogen metabolism.

Constitutional Effects.—The local inflammatory reactions produced by x-rays and radioactive substances in those engaged in such work are

now well known. The action of x-rays may also result in the development of cancer, even with metastases (Tyzzer and Ordway). The more acute constitutional effects of radiations have also been the subject of research.

Edsall and Pemberton have described a toxic constitutional reaction following exposure to x-ray and advanced a theory which they believe to be the basis of this reaction, that is, that the tissue destruction accomplished by Roentgen rays involves chiefly tissues rich in nucleoprotein. The decomposition products of this form of protein are especially rich in substances that are more or less toxic and difficult to metabolize and excrete. The intoxication does not seem to be dependent directly upon alterations of the excreting power of the kidneys because examinations of the urine of two patients showed no evidence of retention. It is probable, however, according to the view of Edsall and Pemberton that in many cases after a time the kidneys do become overtaxed by the added labor thrown upon them and their excreting power fails to a greater or lesser degree and this may increase the toxic symptoms.

Hall and Whipple suggest that Roentgen ray intoxication is due to a disturbance in protein metabolism. They have produced this in dogs by deep massive doses of hard Roentgen rays. The dogs were given lethal doses of x-rays and showed remarkably uniform and constant general constitutional reaction. There was usually a latent period of twenty-four hours or longer when the dogs appeared perfectly normal. After this there were vomiting and diarrhea; death usually occurred on the fourth day. Upon post-mortem examination the spleen of these animals was small and fibrous; the intestinal mucosa was congested and mottled and there was evidence of epithelial injury. The crypts occasionally showed invasion of polymorphonuclear leucocytes. The epithelium showed remarkable speed of autolysis. The authors believe that this injury to the small intestine explains the general intoxication. They find no support for Roentgen ray anaphylaxis or hypersensitiveness to a second properly timed exposure, but there was on the other hand some evidence of a slightly increased tolerance to a second dose. There was no evidence of a Roentgen ray nephritis. The severity of the constitutional reaction was greatly increased by widening the spark gap. The long, latent period, even three weeks, was not explained by these investigators.

Dennis and Martin in experiments on rabbits limited the exposure to various areas of the body and found that toxic constitutional reactions were produced only in animals exposed over areas in which some portion of the intestine was included. Even those rabbits exposed over areas containing only a small portion of the intestinal tract developed toxic symptoms after a rather long latent period, while a particularly severe reaction followed radiation over an area which contained none of the viscera other than portions of the intestinal tract. The animals radiated

over the thighs, the neck and chest continued in good condition and showed absolutely no symptoms although kept under observation for a period of several weeks. It seems to these authors, therefore, tending to confirm the opinion of Hall and Whipple, that injury to the intestinal epithelium plays no small part in the systemic reaction following exposure to roentgen rays. Denis and Martin have suggested also that the reaction after exposure of the abdomen may be due, in part at least, to acidosis on the basis of a lowering of the alkaline reserve, since the administration of sodium bicarbonate by mouth for twenty-four hours following exposure serves to ameliorate or prevent the constitutional symptoms in many instances.

Strauss in a study of the local reaction due to x-rays concludes that there is no real idiosyncrasy but a lessened local resistance in some cases.

Various general symptoms such as headache, malaise, weakness, undue fatigue, unusual need of sleep, fretfulness, irritability, disorders of menstruation, attacks of dizziness have been said by Gudzent and Halberstaedter to be caused by repeated and long continued exposure to radioactive substances. Ordway(*c*) in a study of the occupational injuries due to radium points out that such symptoms are common in many people at times and as they cannot be accurately and objectively recorded they may have been due to close confinement, tiring routine, lack of outdoor exercises and other causes. The exposures of some of the cases reported, however, were doubtless large, some were engaged in the manufacture of radium apparatus and others in the therapeutic application of radioactive substances. It is therefore probable that certain general symptoms did occur as a result of this exposure.

Mottram and Clark estimated by photographic method the daily amount of radiation received by clinical workers making daily applications of radium. These workers received daily scattered over the entire body about 1.4 per cent of the total radiation received by a patient during a course of treatment for superficial carcinoma.

Because of these constitutional symptoms and the effects of radiation upon the blood forming organs great caution and even frequent alternation of service is necessary for those engaged in the use of radioactive substances.

We have personally seen a profound constitutional reaction in a patient injected intravenously with active deposit. Because of this and the widespread character of the lesions produced great care should be exercised in the internal administration of radioactive substances.

Theories of Action.—Hertwig and his school believe that radiations cause a specific destructive action upon the chromatin of the cells. Swartz considers that the injury to the cells is due to the destruction of the cell lecithin by the radiations. Packard suggested that radiations acted indirectly on the chromatin and protoplasm by activating autolytic en-

zymes. Neuberg(*a*) ascribes the effects of radiation to an inhibitory action of x-rays and radium rays upon the other intracellular enzymes without a corresponding deleterious effect upon the autolytic enzymes. Richards(*b*) maintains that the radiations affect the activity of the various enzymes or ferments; that a short radiation may accelerate the activity and a longer be inhibitive so that life processes are subject to marked changes under the influence of radiation.

Radium emanation according to Bovie(*b*) affects the nucleus in a manner similar to the effect produced by quartz rays. Cell division is inhibited as well as locomotion and ciliary action. He finds no reason to believe, however, that rays are more strongly absorbed in the nucleus than in the cytoplasm nor that the nucleus is more photo unstable than the cytoplasm. The effect upon the nucleus may be due to the more intricate nature of its mechanism and to its inability to undergo rapid recovery from injury caused by radiation. The radiations affect the protoplasm at the place where they are absorbed and the observed physiological disturbances are responses on the part of the organism to its injured protoplasm. Bovie believes that it is the instability of the physiological mechanism rather than the wave length of the radiation used which determines the nature of the physiological effect produced. The effect of course is different if one wave length penetrates deep and the other only affects the surface, but the difference is apparently due to the penetrating power rather than any specific effect of the wave length *per se*.

Kronig and Friedrich agree with Bovie that it is not the quality but the quantity, that is, the total energy absorbed, which produces the biological effect.

II. Light

Light has been used as a therapeutic agent for a number of years and its general action is based largely upon hypothesis. From the principal action outside of the living organism and from the constitution of the latter as well as from its known action upon plants and lower animals a certain amount of speculative theory has been indulged in to explain its action.

Light is composed of different kinds of rays. These rays are explained as transverse electromagnetic vibrations having their origin in the rapidly oscillating electrons whose periods are the same as the periods of the wave motion. These wave impulses travel with the same velocity in free space (about 186,000 miles per second). The different colors correspond to different wave lengths (or more properly, to different rates of vibration) and vary in length from approximately 3.9 to 7.6 ten-thousandths of a millimeter. Waves of a similar character whose lengths fall above or below the limits mentioned are not perceptible to

the eye. Those between 3.9 to 1.0 ten-thousandths of a millimeter constitute ultra-violet light. Those exceeding 7.6 ten-thousandths of a millimeter in length are the infra-red waves. The ordinarily used unit of wave length is the Angstrom unit, equal to one ten-millionth of a millimeter. Another unit frequently used is the micron, $\mu = 0.001$ mm.

It is a general law of photochemical action that only those rays are effective which are absorbed by the substance in which the reaction occurs. Visible light rays are not as a general rule active but may be rendered active by impregnating the tissue or other material with certain substances which in such cases act as the photochemical absorbent or sentitizer. Ultra-violet light rays are active as they are the easiest absorbed.

Experience has shown that light can bring about a variety of chemical changes. Neuberg(*c*)(*d*)(*e*)(*f*)(*g*) observed that the general effect of light acting on organic substances present in animal and plant cells is to produce from carbonyl containing materials aldehyds or ketone compounds, whose reactivity and availability for important synthetic changes are conspicuous. These changes, however, could only be produced by the addition of certain salts such as uranium, mercury, arsenic and manganese which acted as photocatalytic agents. Neuberg and Schwarz have shown that iron salts can act as photocatalyzers. They believe that in the presence of light these photocatalyzers take oxygen from the air and pass it on to the organic light receptors. This photocatalytic light action consists in oxidation and cleavage processes. From their investigation they conclude that sensitiveness to light is increased by giving mineral waters containing heavy metals. Pincussohn(*c*) has reported that a solution of sodium urate, containing eosin, exposed to light shows a diminution in the content of uric acid. The proteins of egg white and of the crystalline lens exposed to ultra-violet light were found by Chalupechy to be considerably altered. The albumins were decreased, the globulins increased and some coagulated protein was formed.

The action of light energy on tissues and skin has been studied quite extensively. Bering sums up the work previous to 1914. He states that the action of light manifests itself in cell destruction produced through direct destruction or by edema and thrombosis as a result of a direct action upon the endothelial membrane and musculature of the vessel wall. There also results a hemorrhagic inflammation which terminates with a productive connective tissue formation. The histological changes were almost exclusively produced by ultra-violet light rays. The blue rays possessed only a slight action and the green, yellow and red rays produced no change. Sensitizing of tissues with substances such as eosin increased the action of light but slightly.

Schanz(*a*) has observed that light may alter the cell proteins, especially in the presence of organic and inorganic substances such a silicates, sugar, lactic acid and urea which act as sensitizers. The pyknosis and hyaline de-

generation of cells resulting from influence of ultra-violet light rays are believed by Krebich to be caused by the proteins being rendered insoluble, and as a consequence the catalase is more firmly bound and inhibited in its action. Burge(*d*) believes that ultra-violet radiation kills cells and tissues by changing the protoplasm of the cells in such a way that certain salts can combine with the protoplasm to form an insoluble compound or coagulum. He found the effective region of spectrum to be from 0.254 μ to 0.330 μ. The action of the sun's rays on the non-pigmented skin of animals is ascribed by Beijers to the action of the ultra-violet rays on sensitizing substances which are present in the blood.

The action of light on the blood of animals has been studied quite extensively by Oerum(*b*). He found that the blood volume and the hemoglobin are decreased in the dark. Red light has a similar effect but in blue light a plethora is produced and hemoglobin is increased. Light baths increase the blood volume in the course of four hours about twenty-five per cent. The photodynamic action of light on blood has been reviewed by Bering. By photodynamic action is meant the ability of certain fluorescent substances to produce in light strong biological action. The red blood corpuscles are dissolved, some substances attacking the corpuscles within the cell membrane, in others the primary attack is intercellular. Immune serum loses its specificity. Polynuclear leucocytes and lymphocytes are destroyed. The proteins of serum form a substance having a hemolytic action. Traugott could observe no effect on the number of red blood corpuscles in man following exposure to ultra-violet rays for ten to fifteen minutes. An increase of leucocytes, however, was noted. Another effect observed was that blood coagulated sooner and the number of blood platelets was increased. Schanz(*b*) extended the observation of Chalupechy and studied the effect of ultra-violet light on proteins in the blood and found that after exposure of blood for eight hours there was a decrease in the albumin from 27.0 mg. to 3.9 mg. per 100 c.c. of diluted serum and an increase of globulin from 2.1 to 24.2 mg. per 100 c.c. Hausmann and Mayerhofer noted that salted plasma exposed to ultra-violet light did not coagulate when diluted with water, while untreated salted plasma coagulated in a few minutes. Likewise he observed that oxalated plasma coagulated much more slowly after addition of calcium chlorid when subject to the action of light. From these observations the authors emphasize the necessity of carefully adjusting the action of ultra-violet light upon patients.

The activity of most enzymes is found to be decreased after exposure to light. Agulhon observed that ultra-violet rays may attack enzymes in the absence of oxygen. Chauchard found that the activity of pancreatic amylase is rapidly attacked by rays of wave lengths less than 2800 Angstrom units but not appreciably affected by rays of longer wave length. Lipase was destroyed in part by rays equal to 3300 Angstrom units and

their destructive action increases with decreased wave length, although more slowly than in the case of amylase. The actual percentage loss in activity due to the action of rays less than 2800 Angstrom units is much greater in the case of lipase than in the case of amylase. They could observe no direct relationship between the absorption of ultra-violet rays by pancreatic juice and their action on pancreatic enzymes. Pincussohn noted that the protease activity of the blood of animals injected with a fluorescent substance (eosin) was greater after exposure to light. The rate of destruction of pepsin, trypsin, enterokinase, ptyalin, amylopsin, and the pro-enzyme trypsinogen was reported by Burge, Fischer and Neill to be proportional to the amount of energy applied. The active wave length they used was between 0.302 μ and 0.297 μ.

Metabolism in general is believed to be stimulated by light energy. The experiments of Pettenkofer and Voit(*a*), Johansson, and Lehman and Zuntz show that metabolism with complete muscular rest is slightly greater during the day than at night. Zuntz was first to call attention to the significant fact that even when perfect muscular relaxation ensues there may be still influences such as light on the retina or sounds which may act reflexly on the organism and slightly increase the metabolism.

Cleaves who has reviewed the literature to 1904 concludes that one set of experiments apparently proves that light increases the oxygen carrying capacity of the red blood cells and therefore influences oxidative processes of the organism. Other experiments show increased output of CO_2 when animals experimented on were exposed to light and this increase was supposed to be due to stimulation of the protoplasm, probably due to both stimulation and the increased supply of oxygen. Adult animals therefore fattened more easily in the dark as there is less combustion.

Rubner(*a*) remarks that while the radiant energy of the sun is large in quantity, he has been unable to find any influence upon a man under ordinary circumstances. Zuntz while living on the summit of a high mountain of the Alps observed the basal metabolism increased as much as 40 per cent and that exposure to sunlight was almost without effect on the metabolism. Hasselbalch(*b*) found that if the naked body of a man was strongly exposed to ultra-violet rays the rate of respiration was diminished while the depth was increased. The skin was red with dilated capillaries and the blood pressure fell. Lindhard(*a*), in 1910, showed there is a yearly periodicity of the respiratory rate in the Arctic region, it being less in the spring and summer than in the winter. The enormous variations in the chemical intensity of the sun's rays in the Arctic region are undoubtedly the cause of this effect. The same phenomenon has been observed by Lindhard(*b*) in Copenhagen. The volume of respiration increases 25 per cent in the summer but the intensity of metabolic processes are not affected. While these investigators noted that the ultra-

violet rays of the sun reduce the frequency and increase the depth of respiration, Hasselbalch and Lindhard(*a*) found that exposure to the effect of such rays in the high Alps has no effect upon metabolism.

Animals injected with fluorescent substances such as eosin showed, according to Pincussohn(*b*)(*c*), greatly increased metabolism after exposure to light. The purin bases, amino acids, ammonia and oxalic acid of the urine were increased. Hoogenhuyze and Best have studied the influence of light on the endogenous metabolism of man as indicated by the elimination of creatin and creatinin of the urine. The experimental subjects were put on a creatin and creatinin free diet and normal excretion determined. Following the normal period the subjects were put in a box lined with incandescent lamps for a twenty-minute period and the temperature of the box was 40°-45° C. when closed and 30°-35° C. when ventilated. A series of four experiments showed that exposure to light and heat or to light alone always produced a considerable increase in the creatinin. Creatin was always absent. A negligible effect was produced by exposure to heat alone. A similar increase in creatinin occurred in two patients after a sun bath.

The entire subject of light energy in the physiological relation still calls for careful scientific study and experiment. That light energy influences metabolism is apparently evident by its action on various organic substances of plant and animal origin; by its well-known action on skin and tissues; its action on the blood and enzymes; and by the increased respiratory and endogenous metabolism.

III. Electricity

Various forms of electricity have been used for many years in treating a wide range of pathological conditions but in a very few instances have carefully controlled metabolism studies been made. A literature has grown up among those dealing in electrotherapeutics containing a terminology which is peculiar to this form of medicine. It is for the most part difficult for the scientifically trained physicist to interpret and to estimate dosage accurately in units of electrical measurement. With the active coöperation of competent physicists and clinicians it may be possible to denote measurements, forms and conditions for use of electricity so accurately that the results of metabolic and therapeutic work can be more carefully controlled.

Electricity in various forms is a powerful agent for stimulating nerves and contracting muscles in experimental, diagnostic, and therapeutic procedures. As is well known, death may be caused by electric currents. When these are of low voltage, according to Tousey death is usually due to the production of fibrillation of the ventricles and to interference with

the respiration from the muscular contraction produced. With currents of high voltage there is impairment of the respiratory center. The path of the electrical current through the body and the conditions under which the exposure occurs are variable but very important factors in determining the effect produced.

Electrolysis is commonly used in various conditions for its local destructive effects, notably in the removal of superfluous hair and for the treatment of certain skin diseases such as nevi. A method has been employed known as ionic medication by which certain substances are introduced a varying distance through the skin by means of electrical current.

Hardy in a study of the coagulation of protein by electricity has shown that under the influence of a constant current the particles of protein in a diluted and boiled solution of egg white move with the negative stream if the reaction of the fluid is alkaline and with the positive stream if the reaction is acid. The particles under this directive action of the current aggregate to form a coagulum.

Stewart(*a*)(*b*) has shown that the red blood corpuscles have a very low electrical conductivity in comparison with that of the serum or the plasma and that the conductivity of the blood serum in which the hemoglobin of red blood cells has been dissolved by various methods of laking is increased.

Burge(*a*) has found that in a solution containing both pepsin and rennin the passage of a direct current of ten milliamperes for twenty-five hours results in the complete disappearance of the peptic power, as tested on milk and fibrin, while the action of the rennin is apparently unchanged. In further experiments Burge(*b*) has demonstrated that ptyalin is destroyed by the passage of the direct electric current. This destruction is not due to the electrolytic products; the rate of destruction is uniform, that is, 2.5 per cent per coulomb. The rate of destruction of pepsin by the passage of the direct electric current has been estimated by Burge(*c*) by the decreased amount of egg white digested in proportion to the number of coulombes that were allowed to pass. His conclusion is that the digestive activity of a solution of pepsin is decreased by the passage of the direct electric current at a uniform rate per unit of current. The solutions were kept from polarizing by rapid shaking.

Tousey in his extensive work has described the use of electricity in many pathological conditions. Meyer and Gottlieb in their clinical and experimental pharmacology state that nothing is known about the direct action of electric energy on the metabolic processes of the cells. Steel has reviewed the literature up to 1916 on the influence of electricity on metabolism and concludes that two or more totally different types of electrical currents may have practically the same effect on metabolism. The high frequency type whose action is largely thermic seems to cause an increase in practically the same urinary constituents as the static type whose ac-

tion is largely mechanical, yet it is obvious that the data analyzed is obtained by the work of various investigators under different conditions; particularly to be mentioned is the variation in the amount and form of electrical energy and in the diet of the patients. Steel finds that no extensive metabolic study had been previously attempted and presents the results of his own experiments, using various forms of electricity designated by him as faradic sinusoidal current, directional and autocondensation current with thick dielectric, autoconduction method, the direct d'Arsonval current, combination of direct d'Arsonval current with the autocondensation current with thin dielectric, the static wave current, the galvanosinusoidal current. The special physiological properties of high frequency currents were first published by d'Arsonval(*b*) in 1891.

Steel has shown that relatively strong electric currents of the various types demonstrated caused a stimulation of metabolic processes. The volume of urine is increased by those currents which do not have a pronounced thermic effect and decreased by those currents which have a strong thermic effect and the latter type causes perspiration. All currents increased the total solids, total nitrogen and sulphur of the urine; the most striking and consistent effects were an increase in the urea and creatinin. The greatest increase of urea was obtained with a static wave current and the greatest increase of creatinin with the faradic sinusoidal. Increased elimination of urea was attributed to quickened cellular metabolism and the increased elimination of creatinin to muscular contraction. It is noteworthy that recovery was always prompt and complete in so far as the data indicated. Usually after two days there was no effect. It is important that further study be made of the effect upon metabolism of electrical currents using standard units of physical measurement that can be readily duplicated.

Many patients suffering from a wide variety of conditions undoubtedly derive, at least temporarily, benefit from the various forms of electrotherapeutic procedures yet there is no definite agreement as to the pharmacological action and much more carefully controlled experimental work is necessary before such physical agents as light and electricity, x-rays and radioactive substances can be said to be established in the rational therapy of internal diseases.

Climate

EDWARD C. SCHNEIDER

MIDDLETOWN

The old view which placed the influence of climate upon health above all other factors has very largely been replaced by the view that good hygiene is the all-important health factor. Doubtless careful and intelligent attention to hygiene is more important than climate, and every health seeker should realize that "care without climate is better than climate without care." However, the influence of climate is by no means to be disregarded. The pendulum has swung too far to the side of hygienic living. It must be admitted that even though the health seeker recognizes that the results of following the simple rules of hygiene are restored health, and possibly high efficiency; yet the average individual finds these simple things irksome, and that it requires strength of mind to follow them day in and day out. Climate affects our bodily comforts and causes physiological changes which may play an important part in the curative process. Huntington has demonstrated that human efficiency, as tested by the amount of daily work performed, is determined by physical atmospheric conditions and that the development of the human race is controlled by climate. "Man can apparently live in any region where he can obtain food, but his physical and mental energy and his moral character reach their highest development only in a few restricted limited areas."

Climate, as ordinarily defined, is the resultant of the average atmospheric conditions, considered daily, monthly and annually. It is made up of temperature (including radiation); moisture (including humidity, precipitation and cloudiness); wind (including storms); pressure; evaporation; and also, but of less importance, the chemical, optical and electrical properties of the atmosphere. It is only recently that definite progress in our knowledge of the physiological action of atmospheric conditions has been made. Even now this knowledge is fragmentary; so that medical climatology, which deals with the hygienic effects of climate, is still far from being anything like an exact science.

The physical influences that cause physiological changes are temperature, humidity, air movement and pressure, as met at high altitudes. Light has apparently been found to be a minor factor. The physiological

influence of each of these atmospheric factors will be briefly considered. Pressure will be discussed under altitude.

Temperature and Humidity

Although man is a homothermal organism, there is a certain relationship between his body temperature and the temperature of his environment. His internal temperature, in health, remains fairly constant wherever he may be, varying not more than 1° or 2° F. Man readily adapts himself to extremes of temperature through responses made by his vasomotor system and sweat glands. He is constantly and of necessity eliminating heat. The loss of heat results from radiation, conduction and evaporation. The amount of heat lost by radiation and conduction depends largely upon the temperature of the surrounding air, while the amount lost from evaporation depends upon the relative humidity of his immediate environment. Some conditions permit loss of heat by radiation and conduction only. In a dry hot climate loss of heat by evaporation is at its maximum. The New York State Commission on Ventilation found that during the months of June and July the rectal temperature of man at 8 A. M. was conditioned by the average atmospheric temperature of the preceding night and that a difference of about 1° F. resulted from a difference of 36° F. in atmospheric temperature. The temperature of a chamber influenced the body temperature of healthy human beings, confined for periods ranging from 4 to 7 hours, the body temperature falling in an atmosphere of 68° F. and fifty per cent relative humidity; rising in one of 86° F. and 80 per cent relative humidity; and remaining nearly stationary in air of 75° F. and 50 per cent relative humidity. A stay of three and one quarter hours in an atmosphere of 104.7° F. and 95 per cent relative humidity caused the body temperature to rise 6° F. (25).

Shaklee, working with the native monkey in the Philippine Islands, found that exposure to the sun by placing the animal on the ground or a roof caused death within six hours from a rise in body temperature. It was possible to gradually acclimatize the animals, this being accomplished by an increased capacity for sweating, which kept the body heat well within the killing temperature, although it rose several degrees.

In hot climates radiation and conduction become less important and evaporation the most important factor in eliminating heat. Evaporation in its turn depends upon the relative humidity of the air and, to some extent, upon the presence of winds.

The circulatory system is also affected by the temperature and humidity of the atmosphere, the rate of heart beat being increased concomitantly with the body temperature; it is increased in warm humid air and decreased in cool, dry air. Eastman and Lee found that the pulse

rate increased by 39—from 67 to 106—as the atmospheric temperature rose from 74° to 110° F. and the relative humidity from 58 to 90 per cent. The effect of humid heat upon the blood pressure does not appear to be uniform. Young, Breinl, Harris and Osborne found the systolic pressure rose at times and fell slightly at others. The New York State Commission on Ventilation observed that excessively high temperatures and high humidities were accompanied by an elevation of both systolic and diastolic pressures. The reactions of the vasomotor mechanism, as judged by Crampton's scale of vasotone, indicate that a distinct vascular benefit follows the exposure of the body to a cool dry air.

The influences of atmospheric heat and humidity on the respiration are varied in character. A moderate degree of both seems to be without effect on the rate of respiration; but more extreme rises cause a quickening of the breathing, which is probably accompanied by more shallow respirations. Young and collaborators found that the alveolar air in inhabitants of tropical Queensland showed a lower carbon dioxid content than the European average. A slight seasonal influence has been noticed by Boycott and Haldane, in which a higher alveolar carbon dioxid partial pressure was found in cold and a lower in warm months. These changes were not attributed to variations in the body temperature but to the contact of the body with cold or warm air. A marked increase in relative humidity also lowers the alveolar carbon dioxid content.

The influence of high temperature and high humidity on the capacity for physical work, the amount of blood per kilogram of body weight, and the concentration of sugar in the blood is pronounced. Lee and Scott exposed cats for periods of six hours to an abundance of moving air, varying in respect to temperature and humidity, using a "low" condition in which the average temperature was 69° F. and the humidity 52 per cent; an "intermediate" condition in which the average temperature was 75° F. and the humidity 70 per cent; and a "high" condition in which the temperature was 91° F. and the humidity 90 per cent. Muscles taken from these animals and stimulated to exhaustion showed that the average duration of the working periods and average total amounts of work performed decreased progressively from the low, through the intermediate, to the high condition. The amount of blood taken from the cats was less after exposures to the high than the low condition. The concentration of sugar in the blood also decreased progressively in the three groups from the low to the high condition. The evidence indicates that the distaste for physical labor which is felt on a hot and humid day has a deeper basis than mere inclination; that it is founded upon physiological factors.

Atmospheric conditions likewise influence the nasal mucosa. Miller and Cocks demonstrated that exposure of the body to heat increased the swelling, redness and secretion of the nasal mucosa; and that the effects were more marked when the humidity of the air was high. High tem-

perature with draughts diminished the swelling, secretion and redness; while cold draughts increased these conditions. The effects produced upon the nasal mucosa are direct rather than reflex in nature.

Miller and Noble found that respiratory infection of rabbits was favored by chilling after they had been accustomed to heat. They conclude that the weight of experimental evidence does not justify the elimination of exposure to cold as a possible though secondary factor in the incidence of acute respiratory disease. A change from low to high temperature has even a more marked predisposing influence than that from high to low.

Environmental temperatures likewise exert an influence upon the metabolism of men. Voit(*e*) subjected fasting men to many different temperatures, in the Pettenkofer-Voit respiration apparatus, while he determined the carbon dioxid and nitrogen elimination. Changes in temperature from 57° to 80.6° F. scarcely changed the carbon dioxid output; a lowering of temperature to 50° and less stimulated the metabolism; also above 80.6° it was markedly increased, as shown by the rise in carbon dioxid elimination. These observations on man are similar to metabolic changes recorded by Rubner(*j*) for the dog and other animals. Rubner has shown that increased humidity at temperatures above 82° F. increases the metabolism. For a given high temperature the rise in metabolism will not be as great where the evaporation of perspiration occurs readily as when there is difficulty in evaporation, due to increased humidity, that prevents effective elimination of heat.

All studies on the influence of temperature and humidity indicate that cool and comfortable atmospheres, with a temperature of about 68° F. and 50 per cent relative humidity are beneficial; while a temperature as high as 86° F. and 80 per cent relative humidity are deleterious. The bad effects are due primarily to the inability of the body to properly cool itself because of the temperature and moisture conditions of the surrounding air.

Air Movement and Winds

Here again the gain to the body is to be found chiefly in the influence of moving air on heat loss. The air surrounding the body soon becomes saturated with moisture and approaches the body heat in temperature. Hence this thin envelope of air surrounding the body may establish the degrees of temperature and humidity that are known to be deleterious.

The effect of wind of moderate humidity and different temperatures on the metabolism of a man clad in summer clothes as compared with the metabolism in calm air was shown by Wolfert(*b*) to be stimulating. A breeze having a temperature of 59° to 68° F. and moving at the rate of

about 15 miles per hour increased the metabolism approximately 19 per cent.

A recent investigation by Aggazzotti and Galeotti on the influence of wind on the respiration and the pulse has shown that if the wind is not too strong the lung ventilation is favored. The alveolar carbon dioxid tension is lowered. In strong wind the breathing shows irregularity in rate and depth.

Light

The opinion has been held that the intense light of the tropical skies causes the backwardness of mankind in these countries. Sun baths have been employed in the treatment of tuberculosis with some degree of success. However, the physiological effects of light have not been clearly demonstrated. Wohlgemuth, in a study of desert climates at Assuan, found the number of red corpuscles and the per cent of hemoglobin to be slightly increased. That the increase was not the result of the loss of water from the blood because of sweating was shown by the observations that neither the sodium chlorid nor the sugar content of the blood was changed. He attributes the increase in red corpuscles, which in one man rose from 4,900,000 to 5,680,000 in five months, to the action of light; and cites that Bickel, on exposing rabbits to the light of the mercury arc, produced an increase in the red corpuscles. Other possibilities were not eliminated. Huntington, in his investigation on human efficiency, as measured by the amount of daily work performed, found that the effect of light was at best only slight.

Rubner, under ordinary conditions, and Durig and Zuntz, on Monte Rosa, did not find that sunlight influenced metabolism. Hasselbalch and Lindhard(*a*), studying the ultra-violet rays of the sun, obtained no effect upon the metabolism. They did, however, find a reduction in the frequency and an increase in the depth of respiration as the effect of the exposure to such rays.

The importance of climatic conditions in the life and efficiency of mankind has been well demonstrated by Ellsworth Huntington in his book on "Civilization and Climate." He points out that for the production of good fruit the three factors of good stock, proper cultivation, and favorable climatic conditions are absolutely necessary. Recognizing the importance of these three for man, he then proceeds to study conditions of human progress and power of achievement. He finds that wherever civilization has risen to a high level, the climate appears to have possessed those qualities which to-day are recognized as most stimulating. He derives the important climatic factors by various statistical comparisons. Assuming that the best and fullest test of efficiency is a person's daily work, the thing to which he devotes most of his time and energy, he

studies the output of thousands of industrial workers in various parts of the United States; mental activity of certain classes at West Point and Annapolis; and strength tests of school children in Denmark. The annual work curves are quite similar. The lowest period of efficiency occurs in December, January and February, reaching the minimum at about the end of January. The efficiency curve then gradually rises to a first maximum in May and June, falling moderately until the end of July, rising again in September, with the greatest maximum in November. He also presents a curve of gain in body weight based on a report of patients suffering from tuberculosis in a sanatorium at Saranac Lake. This is similar to the work output curve with the least gain or no gain in February and March, and the maximum gain in October. A study of death rate reveals another of the same type of curves, a marked reduction in May and June, an increase in July and August; followed by another reduction in which the low death rate occurs in October, November, and December, with November showing the lowest rate for the year. All these data combine to demonstrate that the period of greatest physical and mental efficiency occurs in the late spring and late autumn.

An analysis has convinced Huntington that changes in the barometer, in the localities studied, seem to have little effect. Humidity possesses a considerable degree of importance, but the most important factor is clearly temperature. He came to the conclusion that the optimum temperature of outside air for physical well being is from 60° to 65° F., that is when the noon temperature rises to 70° F. or even more; and for mental work the optimum is reached when the outside temperature averages 38° F. Another highly important climatic condition is that of the temperature change from day to day. "It seems to be a law of organic life that variable temperature is better than uniformity." The ideal conditions are moderate temperature changes, "especially a cooling of the air at frequent intervals." Variations in temperature give one of the best tonics provided by nature.

All experimentation and observation go to demonstrate that climate exerts a noteworthy influence on the physical and mental life of mankind. This effect is largely due to the movement, humidity and temperature of the air. Another physical factor, altitude, is still to be discussed.

The Psychological Factor in Climatotherapy

The principles of climatic treatment are founded on psychology as well as physiology. The external conditions which we see and feel make a greater conscious impression than the physiologic effects which do not come into the field of consciousness; unless, as is rarely the case, they are extreme and unusual. A climate that is conducive to out-of-door living

awakens an interest and zest and produces a cheerful serenity and happiness that permit the physiological climatic effects to more completely restore health. Unquestionably both physiological and psychological conditions influence physical well-being; a patient worried about financial resources and family cares rarely secures the full advantage of the physiological effects of climate, because of the absence of serenity and cheerfulness.

The only way to use a climate is to give it every chance to help in the cure. Careful and intelligent attention to personal hygiene and to the psychical side of the environment are essential. Climate does not cure, but it is an important help to the body in overcoming weakness and disease.

The Variety of Climate.—The physical factors have served as a basis for classifications of climate. It has long been recognized that there are four factors that enter into the production of the climate of any locality: (1) Distance from the equator; (2) distance from the ocean; (3) height above the sea-level; and (4) the prevailing winds.

The classic zones, tropical, temperate and polar, recognize the relation to the sun and are based on sunshine distribution. Irregularities in the distribution of land and water and the prevalence of particular winds break the uniformity of these zones and lead to a more rational scheme of classification. "The great differences in the climatic relations of land and water, recognizes a first large subdivision of each zone into land and water areas. Then as continental interiors differ from coasts, and as windward coasts have climates unlike those of leeward coasts, a further natural subdivision would separate these different areas. Finally, the control of altitude over climate is so marked that plateaus and mountains may well be set apart by themselves as separate climatic districts."

A maritime climate is equable, that is without extremes of temperature, with a prevailing high relative humidity, a large amount of cloudiness and a comparatively heavy rainfall. The continental climate is more severe; the annual temperature ranges increase, as a whole, with increasing distance from the ocean; the regular diurnal ranges are also large, reaching 35° or 40° F., and even more. The humidity is lower and cloudiness, as a rule, decreases inland, reaching its minimum in the arid plains and deserts. The evaporating power of a continental climate is much greater than that of the more humid and cloudier coast climate. A climate with a relative humidity up to 50 per cent is unusually dry, with 50 to 70 per cent relative humidity is dry, with 70 to 85 per cent relative humidity is moist, and with 85 to 100 per cent relative humidity is unusually moist.

General Considerations in the Choice of Climate.—While climatic studies are difficult to evaluate certain things now stand out somewhat clearly. The humid tropics are disagreeable and hard to bear. Energetic physical and mental actions are difficult or even impossible. "The monotonously enervating heat of the humid tropics weakens, so that man becomes

sensitive to slight temperature changes." James is of the opinion that an even temperature lowers the tone of the vasomotor system by lack of proper exercise. In drier tropics, cooled by trade winds, as found in the Hawaiian Islands, the white population lives and carries on business in "American style" without signs of tropical enervation and deterioration. It appears that many elderly persons and others who are overworked may find rest from nervous tension in portions of the tropics.

Extraordinarily low temperatures are easily borne if the air is still and dry, and large ranges in temperature are well tolerated when the air is dry. On the other hand, cold air with a high moisture content has a depressing effect. At the margins of the polar zones the change from winter to summer is so sudden that the transitional season disappears. Hence, in the seasonal changes the intermediate periods that add so much to human efficiency are lacking.

It has been suggested that unless invalids are of very delicate constitution, or greatly run down in health, the bracing qualities of a northern winter in a dry climate under proper safeguards will probably do them more good, though at times they will be less comfortable, than a warm southern atmosphere. Too large variations of daily temperature may be overtrying, but as a rule a definite drop in the daily temperature is a necessity for stimulation.

Altitude

The mountain and high plateau are characterized by a similar climate in all the geographical zones. The characteristics are decrease in pressure, temperature and absolute humidity; an increase in the intensity of sunlight and radiation; and larger ranges in soil temperature. The climatic action of the heat, humidity and light have been discussed, leaving only the factor of pressure for consideration. Some maintain that the real benefit of mountain climate to the health seeker is to be found in the favorable heat and humidity and the mental reaction to the beauty of the environment.

An early suggestion made by Jourdanet is still to be borne in mind when mountain and high plateau climates are recommended. He divided these climates into the mountain climate, below 6,500 feet, and altitude climate above that height. The former was considered beneficial because of the stimulating quality of clean, clear, cool air and the latter injurious because of low pressure. Men live comfortably and work well in the mines of the Andes at 15,400 to 16,200 feet. Such altitudes, however, are for the robust and not the health seeker.

Residence at a high altitude brings about striking and definite physiological changes in the body. There have been many opinions held as to the essential cause. A common belief has been one that regarded the

pressure, acting in a mechanical manner, as the responsible cause. It has been natural to expect that a diminution of external pressure would have a "cupping glass" effect that would lead to a congestion of the skin and lungs and in some way cause a readjustment of internal parts of the body. However, all recent investigators hold that the physiological effects noted at high altitudes are due to the lack of oxygen, resulting from the lowered partial pressure of oxygen that occurs proportionately with the decrease in barometric pressure.

Altitude Sickness.—It is now clearly established that during the first few days spent at a high altitude an attack of altitude sickness may occur. Some persons are affected at a comparatively low and others at a higher altitude. An elevation of 10,000 feet, or even less, provokes it in a few individuals; but many go to 14,000 and more feet without distress. There are two forms of altitude ("mountain") sickness; the acute, which breaks out suddenly on entrance into the rarefied air; and the slow, which manifests itself much later.

The acute form is characterized by a rapid pulse, nausea, vomiting, physical prostration which may even incapacitate for movement, livid color of the skin, ringing sensation in the ears, dimmed sight and fainting attacks.

In the slow form, which may be called the normal type, lasting from one to three days, the newcomer at first complains of no symptoms. Some hours later he begins to feel "good for nothing" and disinclined for exertion. He goes to bed to spend a restless and troubled night. A frontal headache and periodic or Cheyne-Stokes breathing interfere with sleep, there may be nausea and vomiting. The next morning the patient may feel slighly giddy on arising and any attempt at exertion increases the headache. The face may be slightly cyanosed and the eyes dull and heavy, with a tendency to water. The tongue is coated and appetite gone. There may be diarrhea and abdominal pain. The pulse and arterial blood pressure are usually high. The temperature is normal or slightly under. There are wide divergencies from this slow type of which Ravenhill has well described those in which cardiac and nervous symptoms predominate. A weakened heart does not seem to predispose to the cardiac type of altitude sickness.

Acclimatization.—The process of acclimatization is slow, while certain of the changes may begin almost at once with entrance into rarefied air, it ordinarily requires several days for these to wholly restore the patient to normal well being. The complete process of acclimatization requires six and more weeks.

Adaptation to altitude consists in physiological responses that increase the supply of oxygen, which is at first decreased because of lowered pressure, until it again reaches normal. These include, among others, the following: (1) An increase in the percentage and the total amount of

hemoglobin in the blood of the body; (2) a fall in the lung alveolar carbon dioxid partial pressure and a rise in the alveolar oxygen pressure, the result of increased ventilation of the lungs due to deeper breathing; and (3) at some altitudes a temporary or permanent increase in the rate of blood flow.

The Blood Adaptive Changes.—In spite of an occasional contrary observation the prediction made by Paul Bert in 1878 that the blood at high altitudes would be found to have a greater oxygen capacity than the blood of similar individuals at lower levels, has been demonstrated to be true. Investigators have found an increase in the number of red corpuscles per cubic millimeter and in the percentage of hemoglobin. Miss Fitzgerald(*a*)(*b*), by a study of inhabitants of the Southern Appalachian and the Rocky Mountains, found that as the altitude increases the percentage of hemoglobin in the blood is augmented about 10 per cent of the normal value, for men and women at sea level, for every 100 mm. fall of barometric pressure. The physiological significance of this increase in hemoglobin and red corpuscles is that a unit volume of blood can carry for a given oxygen pressure more oxygen than normally.

When a rapid ascent is made to a high altitude, as in an aeroplane, the changes in the blood may be detected as early as in from 20 to 60 minutes. When the ascent is made more slowly, as by automobile or railway, it may not be evident for 12 or more hours. The increase is rapid for the first two to four days and is followed by a more gradual increase extending over a period of six weeks. The increase occurs most rapidly in subjects in excellent physical condition. Fatigue, as from walking up a mountain, delays the increase in hemoglobin and red corpuscles.

At the present time the evidence accounts for the increase in hemoglobin and erythrocytes as follows: the initial rapid increase is due to a concentration by a loss of fluid from the blood and possibly by throwing into the general circulation a large mass of reserve corpuscles. The more gradual increase, extending over several weeks, is brought about by the increased activity of the bone marrow resulting in an increase in the total number of corpuscles and amount of hemoglobin which may finally not only restore, but sometimes actually increase, the low altitude blood volume.

The number of leukocytes per cubic millimeter is not increased with altitude, but the larger lymphocytes are increased and the polymorphonuclear cells diminished. The blood platelets are also increased at high altitudes.

Respiratory Adaptation to High Altitudes.—The first effects observed on going to a high altitude are caused by an insufficient supply of oxygen to the tissues. It is to be expected, therefore, that the amount of air pumped in and out of the lungs will be increased almost immediately.

The respiratory response to altitude is ordinarily the first of the several compensatory changes to appear. Miss Fitzgerald found that the breathing of persons living permanently at an altitude of 2,200 feet, as indicated by the alveolar carbon dioxid, showed a larger lung ventilation than under similar conditions at sea level; and further established the law that approximately a 10 per cent increase in the ventilation occurred for each 100 mm. of diminution of the barometric pressure. The full extent of the change in breathing is reached in from 7 to 14 days.

The type of breathing that is best suited to the need of the body at high altitudes is slow and deep rather than rapid and shallow. After adaptation the depth rather than the rate of breathing will ordinarily have increased. However, during vigorous physical exertion, where even at sea level the depth of breathing is about maximal, at a high altitude such as Pikes Peak the rate shows a marked increase. A subject, who had breathed when in bed at sea level at the rate of 16.8 breaths per minute, on Pikes Peak had a rate of only 17.3; while walking, at the rate of 5 miles per hour at sea level, the rate was 20, and on Pikes Peak 36 breaths per minute.

The increased breathing augments the alveolar oxygen tension in the lungs. If, for example, on Pikes Peak, with a barometric pressure of 457 mm., the respiration did not change, then the alveolar oxygen tension in the dry alveolar air would fall proportionately with the barometer to about 36 mm. The increase in breathing, however, raises this at that altitude to about 52 mm. As a result the blood will be just that much more saturated with oxygen, thus remedying to some extent the defective saturation of the arterial blood with oxygen.

The explanation of the manner in which respiration is modified has recently been more fully elucidated. The hormone of breathing is the hydrogen ion concentration in the blood, and not the total carbon dioxid in the blood, nor the concentration of HCO_3 ions as has sometimes been claimed. Haldane(*d*) has pointed out that the H-ion concentration of the blood is regulated with great delicacy by the respiration on the one hand and the kidneys and liver on the other. The respiration doing the rough and immediate work by increasing or decreasing the elimination of the carbon dioxid, and the kidneys the finer and slower work. When a person goes to a high altitude the want of oxygen acts as an additional stimulus to the respiratory center with the result that an excess of carbon dioxid is eliminated. This decreases the H-ions and causes a state of alkalosis in the blood. To offset the excess of alkali the kidneys and liver attempt to redress the balance. It has been shown by Haldane, Kallas, and Kennaway and by Hasselbalch and Lindhard(*a*)(*b*) that excretion of acid and of ammonia diminish for a period of several days. During this time the alkalosis will have been diminished and the normal H-ion concentration of the blood almost restored to its previous level. This, as Haggard and

Henderson(*a*) have shown, results in a reduction of blood alkali. While after acclimatization the H-ions are again probably nearly the same as at sea level, the restoration is never complete and in the end the stimulating action of diminished oxygen leads to a greater ventilation of the lungs than on the first day, and a permanent level is then established for that barometric pressure. Haldane makes clear that if the initial alkalosis should be maintained the dissociation of oxyhemoglobin would be less than normal, thus accentuating oxygen want in the body. By restoring, or nearly restoring, the H-ion concentration of the blood the curve of oxyhemoglobin dissociation is again shifted back to or toward the normal for sea-level.

Metabolism.—Investigations, in spite of an occasional positive finding, lead to the opinion that metabolism is independent of the variations in atmospheric pressure. Sundstroem found that the assimilative power for the energy in the food remains normal at all altitudes.

In 1883 Fraenkel and Geppert placed a fasting dog under the influence of diminished barometric pressure and found an increased protein metabolism. Zuntz(*a*) and collaborators, on Monte Rosa at 2,900 m., failed to show an increase in metabolism; but at 4,560 m., barometer 443 mm., obtained an increase of approximately 15 per cent. Later Durig and Zuntz, in an expedition to Teneriffe, altitude of 3,160 m., failed to show an essential difference in metabolism. The Anglo-American expedition to Pikes Peak found no difference in metabolism either during rest or when taking exercise. Hasselbalch and Lindhard observed a man for 14 days in a pneumatic cabinet, at 455 mm. barometric pressure, and found that the consumption of oxygen and the urinary ammonia and amino-acids were unaffected. Sundstroem showed that the iron balance did not alter nor the retention of iron exceed that observed in low altitudes.

The diminished excretion of ammonia observed by Hasselbalch and Lindhard and by Haldane and collaborators during the period when blood alkalosis was being overcome has already been pointed out. Hasselbalch and Lindhard found that an increased oxygen consumption might occur during the process of acclimitization. Von Wendt(*b*) noticed a retention of nitrogen, iron and potassium on Monte Rosa which he attributed to the construction of new red corpuscles.

The Circulatory Mechanism.—Altitude, if great enough, increases the heart rate; but it is generally recognized that at moderately high altitudes, 6,000 to 8,000, or even 9,000 feet, there is no augmentation. Shortly after ascending to such an altitude as 14,000 feet the heart rate gradually increases during a period of several days. In persons who develop "altitude sickness" and in those fatigued by climbing, the acceleration begins sooner and is greater. With the development of acclimatization the heart rate will return toward, and in some cases reach, the low

altitude normal. The same amount of physical exertion increases the pulse rate more at a high than at a low altitude. The difference becomes greater as the amount of work done increases.

The arterial blood pressures are not altered by altitude in the majority of men; but in a considerable number of cases there occurs a slight lowering of the systolic pressure; while occasionally, very likely in a poor reactor, there is a rise in both the systolic and diastolic pressures. During an attack of "altitude sickness" there is usually a marked increase in both pressures.

The blood pressure in the capillaries is either unchanged or less than at sea-level. In the veins, at altitudes of more than 6,000 feet, the pressure is less than at sea-level. Contrary to common opinion bleeding from the nose, lips, lungs, and stomach rarely occurs. The experience of aviators has dispelled the belief that altitude causes hemorrhages.

Physical exertion makes greater demands on the heart and blood vessels at high than at low altitudes. The rise in arterial pressure is greater for a given exertion at a high than a low altitude, the difference being less after acclimatization. It would be an easy matter to seriously injure the heart during the early days of residence at high altitude. However, in men who are physically strong because of athletic training the risk is slight; and in all who become acclimated the ordinary forms of exercise will be well tolerated.

General Considerations.—Anemia is regarded by Sewall as the dominant disorder at high altitudes. Anemia reduces the working efficiency and the reserve power of the tissues insofar as it permits deprivation of oxygen. That the physiological response to the stimulation of lowered barometric pressure may be slow or deficient is a common observation. Hence it is to be expected that many functional disorders are originated or accelerated at moderate altitudes owing to the existence of comparatively mild grades of anemia. Moleen has called attention to the fact that individuals who exhibit nervous symptoms or complain of "nervousness" while living at high elevations show a relative or absolute anemia. It is significant that the plethoric type of person rarely finds it necessary to leave high altitudes for "nervousness." It is maintained that if measures are taken to stimulate the blood forming centers there is no more difficulty in living tranquil lives in the high altitudes than at sea level.

The dangers to the heart in high altitudes are, according to Hall, precisely the same as elsewhere, but very sharply exaggerated in certain directions; particularly because the newcomer is likely to overdo in physical exertion. Cardiac overstrain from exercise is often the real cause of distress and not the altitude. Schrumpf found in Switzerland that up to 7,000 feet pathological blood pressures are improved, that is, high pressures are reduced and low ones increased, together with an improve-

ment in the general condition. Compensated valvular lesions and mild cases of myocarditis were also favorably influenced.

Because the adaptive compensations to high altitudes are slow in their development, the newcomer should remain quiet for a day or two. If symptoms of "altitude sickness" occur rest in bed with windows open is advisable and at least a day of quiet after all symptoms have disappeared. During the first days it is best to make no exertion which causes any considerable dyspnea.

The changes in the breathing and the blood are permanent in character, and do not diminish during a protracted residence at the high altitude. Changes in pulse rate and in the rate of blood flow are less permanent, and tend to disappear with acclimatization. On returning from a high to a low altitude the changes in the respiration and blood are maintained for a time as an "after effect." The longer the residence at the high altitude the more prolonged the period of "after effect." During this period the individual may gain in weight and health.

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