Biochemistry of Plants and Animals

BIOCHEMISTRY

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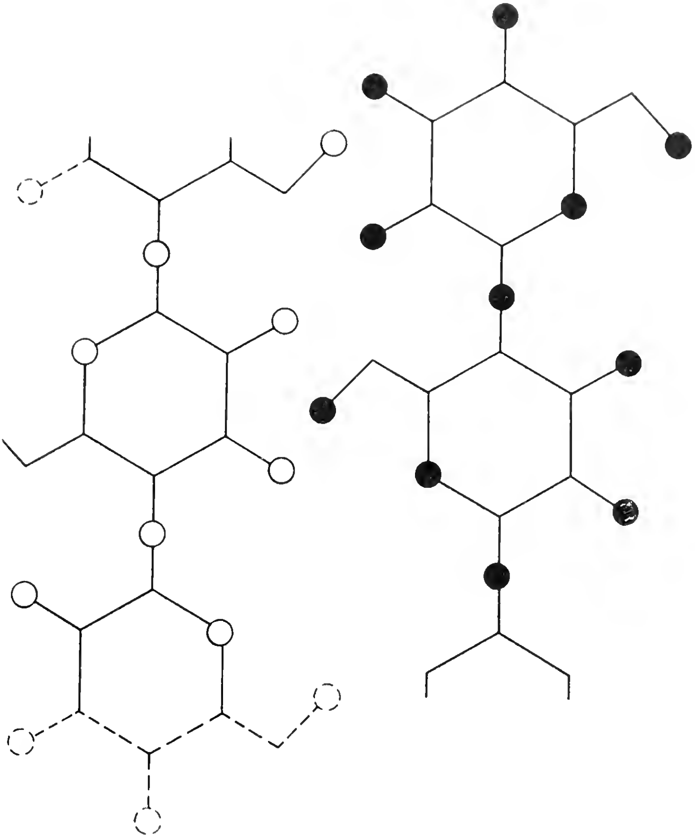

# of PLANTS and ANIMALS

AN INTRODUCTION

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To Our Wives
RUTH, VIRGINIA, and MARY

Preface

In common with many other fields of study, biochemistry has expanded rapidly in the last decade. Still more recently there has been a general trend to both more intensive and more widespread collegiate training in science. Therefore, our knowledge of biochemistry and our concept of what phases should be taught have changed markedly. All participants in this "revolution" agree that change will continue and will probably accelerate.

Although this book is essentially new, it originated from *Introduction to Agricultural Biochemistry* by Dutcher, Jensen, and Althouse, published in 1951. In turn this latter book derived from another of the same name by Dutcher and Haley (1932). We are deeply indebted to these earlier authors for the inspiration and guidance they have given us.

We have rewritten the text completely, endeavoring to broaden the coverage of underlying basic information, to raise the level in keeping with the better backgrounds of students now entering the subject, and to describe briefly some of the great advances being made in the field. At the same time a textbook must be relatively short, requiring a compromise on coverage. Therefore, we have sharply restricted the material on the historical development of biochemistry and the discussion of soils. Insufficient space also has prevented consideration of farm chemurgy and pesticides.

The book is divided into three major sections, devoted to general biochemistry, plant biochemistry, and animal biochemistry. Individual chapters are placed in these sections according to the orientation given the material. It is obvious that any such classification is arbitrary in specific cases. In the interests of brevity we have attempted to make suitable textual reference to appropriate sections rather than redevelop the subject in the additional context.

Basic training in inorganic and organic chemistry is assumed in students using this book. Although it is intended to provide a general knowledge of biochemistry for students in the agricultural sciences, an effort has been made to orient the treatment broadly enough for elementary courses intended for students from other disciplines. As is customary in textbooks, most chapters are relatively complete units permitting omissions in conformance with the lengths and needs of particular courses.

We are indebted to Anita Zellers and Janet Powlus for typing the manuscript.

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University Park, Pennsylvania
May, 1960

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GENERAL BIOCHEMISTRY

part 1

The development of agricultural chemistry

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When we attempt to study the factors that have played important roles in the development of scientific agriculture, we find that chemistry has occupied a most prominent place. The part that chemistry has played in this development has been of such far-reaching importance that a special branch of this science, known as agricultural chemistry, has been a natural outgrowth. It is to this particular phase of chemistry that we wish to direct the reader's attention, for agricultural chemistry, probably more than any other single factor, has been responsible for the development of the quantitative aspects of modern agricultural practice and for the elimination of the old "rule-of-thumb" methods which had been followed for centuries.

BEGINNINGS OF AGRICULTURAL SCIENCE

Nearly all the early workers who were interested in solving nature's secrets as they relate to agriculture were trying to discover "the principle of vegetation." They were seeking to answer the question, "Why and by what method do plants grow and develop?" One of the first theories which aimed to explain the secret of plant growth was that advanced by a Belgian physician and alchemist by the name of van Helmont. Working in the latter part of the sixteenth and the early part of the seventeenth centuries, he was among the first to introduce the use of the balance and to interpret data from the quantitative standpoint. It should be remembered that water was one of the recognized chemical elements at the time of van Helmont's work, and as a result of his studies he concluded that water must be the "principle of vegetation," citing the following experiment as proof of his theory that water could be transformed into plant tissue:

> I took an earthen vessel in which I put 200 pounds of soil, dried it in an oven, then I moistened it with rain water, and pressed hard into it a shoot of a willow weighing 5 pounds. After exactly 5 years the tree that had grown up weighed 169 pounds and about 3 ounces. But the vessel had never received anything but rain water or distilled water, to moisten the soil when this was necessary, and it remained full of soil which was tightly packed, and lest any dust from the outside should get into the soil, it was covered with a sheet of iron coated with tin, but perforated with many holes. I did not take the weight of the leaves that fell in the autumn. In the end I dried the soil once more and got the same 200 pounds that I started with, less about 2 ounces. Therefore, the 164 pounds of wood, bark, and roots arose from the water alone.

This experiment is thoroughly typical of much of the early investigational work in agricultural chemistry, as well as of other sciences. In this, as in other branches of science, it is very easy to fail to consider a vital factor and, as a result, to draw from perfectly good experiments a conclusion which appears to be correct but which is in reality entirely wrong. In the work cited above, van Helmont failed to take into consideration two most important factors, namely, the role played by the constituents of the atmosphere, and the small amount of soil which had disappeared.

Some years after van Helmont reported his result, Glauber proposed the hypothesis that saltpeter is really the "principle of vegetation." This conclusion was reached by Glauber because he secured such large increases in the yield of crops by applying this material as a fertilizer. For many years his view was widely accepted by agricultural writers. The only prominent opponent was Jethro Tull, who believed that the fineness of the soil particles had a beneficial influence on plant growth. According to this latter view, it was "the very minute particles of soil loosened by the action of moisture that constituted the proper 'pabulum' of plants. The pressure caused by the swelling of the growing roots forced these particles into the lacteal mouths of roots where they entered the circulatory system. All plants live on these particles, that is, on the same kind of food." Various other ideas regarding the "principle of vegetation" were proposed. The general view held at the close of this period cannot be better summed up than in Tull's own words: "It is agreed that all the following materials contribute in some manner to the increase of plants, but it is disputed which of them contributes most to that increase of food: nitre, water, air, fire, and earth."

During the latter half of the eighteenth century a considerable interest was manifested in all phases of agriculture. Textbooks were written, experimental work was stimulated, and societies were formed for the promotion of agriculture. In 1755 the Edinburgh Society of England employed a chemist by the name of Francis Home "to try how far chemistry will go in settling the principles of agriculture." Home, believing that the whole system of agriculture was dependent upon plant growth, prosecuted his research along the lines of plant nutrition, finally drawing the conclusion that there were at least six plant-food materials: air, water, earth, salts of different kinds, oil, and "fire in a fixed state." After the work of Home there was no important advance in agricultural chemistry for 40 years. From what has been stated, it is evident that anything like an adequate idea of the growth and composition of plant bodies could not be obtained until certain of the important chemical elements had been discovered and the composition of water and other common substances had been established.

During the period 1770 to 1800 some of this necessary work was accomplished, but its importance in agriculture was not appreciated at the time. This work, the discovery of oxygen by Priestley and by Scheele, the discovery of the composition of water by Cavendish, and the explanation of combustion by Lavoisier, served to open the way for the development of what we consider modern chemistry.

After the work of Home further progress in scientific agriculture was hardly possible until greater use was made of accurate chemical methods of investigation. Although Ingen-Housz receives credit for the discovery of the role that carbon dioxide plays in plant economy, it remained for De Saussure to place this discovery on a firm scientific basis by the use of quantitative methods. His book, *Researches upon Vegetation,* published in 1804, was really the first scientific work showing the source of the carbon compounds in plants. He established, by means of quantitative experiments, that the increase in the amounts of carbon, hydrogen, and oxygen, when plants were exposed to sunlight, was obtained from the carbon dioxide of the atmosphere and the water of the soil. This early investigator also stated that the mineral elements derived from the soil were essential to plant growth. In proof of this point he gave the results obtained for the analyses of the ash of many different plants. He believed that plants obtain the greater part of their nitrogen from the soil. These views of De Saussure have since been investigated and verified by many different scientists and are substantially those held at the present time regarding the fundamental principles of plant nutrition. In the days of De Saussure, however, his opinions were not accepted as true, and it was nearly half a century later that Boussingault, Liebig, and others repeated the investigations of De Saussure and confirmed his results, which then were finally accepted by the scientific world.

Between 1802 and 1812 Sir Humphry Davy delivered lectures annually on agricultural chemistry. These lectures were published in book form in 1813 and entitled *Essentials of Agricultural Chemistry*. This was the first textbook of the modern period; it treated of the composition of the air, soil, manure, and plants, and of the influence of heat and light on plant growth. Although some of the views expressed in this work were incorrect, it was for the most part a carefully prepared summary of the accepted knowledge obtained from the results of previous investigations.

Thaer also published an important work entitled *Principles of Rational Agriculture,* in which he proposed the so-called humus theory of soil fertility. He believed that plants obtain their nourishment from the humus, and consequently it is this material which determines the productivity of the soil. Thaer's idea, however, was shown to be inadequate in accounting for the sources of plant food, and it prevented, for a long time, the recognition of the actual value of humus as a factor in soil fertility.

About 1830 Boussingault became actively engaged in agricultural research and began a series of field experiments on his farm in Alsace. He was the first investigator to have a chemical laboratory on a farm and to make thoroughly practical investigations in connection with agriculture. The establishment of this laboratory marked the start of the first agricultural experiment station. In his work Boussingault is credited with reintroducing the quantitative methods of De Saussure. He also did very important work upon the assimilation of the free nitrogen of the air by plants. He ascertained many important facts relating to the chemical characteristics of foods, made a comparison as to the quantity of nitrogen in different kinds of feeding stuffs, and compared their values on the basis of the nitrogen content. His study on the production of saltpeter did much to prepare the way for later work on nitrification.

In 1840, Justus von Liebig's report to the British Association (published later as *Chemistry in Its Application to Agriculture and Physiology*) stimulated a great amount of discussion in scientific circles. Von Liebig delighted in taking plant physiologists to task for their lack of chemical knowledge and for ignoring the accumulating experimental evidence that plants receive their carbon supply from the atmosphere rather than from the carbonic acid from the soil. Before this time many workers adhered to the humus theory of Thaer. The publications and lectures of Boussingault and De Saussure had made little impression on the proponents of the humus theory. After Liebig's tirade, there were few who dared to oppose his theory of carbon dioxide assimilation. In the first edition of his book Liebig called attention to the fact that farms from which certain products were sold gradually became less productive because of the decrease of nitrogen, but he made one serious mistake. He overestimated the amount of ammonia present in the atmosphere and underestimated the value of nitrogen in the soils and manures. A study of the chemical composition of the ash of plants was also made by Liebig. His results led him to propose the mineral theory of plant nutrition. Previous to this time De Saussure had proved that plants contained certain mineral elements, but he had laid no emphasis upon their importance as plant foods. Liebig's publications on the composition of the mineral substances present in plants and his emphasis on their importance as plant foods led to the commercial preparation of manures, which in later years has developed into the great commercial fertilizer industry.

In 1837 John Lawes began a series of experiments on the application of various manures to potted agricultural plants on his home farm at Rothamsted, England. He soon discovered that to carry on his work in a proper manner he needed the assistance of a trained chemist and engaged the services of J. H. Gilbert, one of Liebig's former students. Thus in 1843 the second agricultural experiment station was established at Rothamsted. It is still in operation at the present time, having been endowed with funds by John Lawes. Many of the Rothamsted experiments have been conducted continuously since 1844, and results of the greatest value to agriculture have been obtained as a reward of the earnest, persistent work of Lawes and Gilbert.

Our modern science of bacteriology is, in a large measure, the outgrowth of the early work in agricultural chemistry. As men studied and worked they found that chemical explanations were not sufficient, and from the earliest times until the present the chemist has made use of all the other sciences, indiscriminately, to aid in the solution of his problems.

The pioneers in agricultural chemistry first confined their attention to the chemical phases of plant growth. Ingen-Housz and De Saussure showed that atmospheric carbon dioxide is the source of carbon in plant compounds. Later Lommel and Pfeffer emphasized the importance of light in the synthesis of organic compounds in the plant, and Sachs, Leow, Baeyer, and others pointed out the important function of the green pigment, chlorophyll, in this synthetic process. It remained for Willstatter and his students to shed further light on the chemical composition of chlorophyll.

One of the earliest chemists who exerted a real influence on American agriculture was Samuel L. Mitchell, who held the chair of chemistry and agriculture at Columbia College from 1792 to 1801. Mitchell helped to found the New York Society for the Promotion of Agriculture, Manufactures and the Useful Arts, and through his efforts considerable interest was developed in the use of gypsum as a fertilizer.

In 1806, Thomas Ewell, of Virginia, published an important treatise in which he stated:

> Agriculture is most intimately connected with chemistry. The power of seed to attract and unite to parts of the soil, so as to vegetate or increase in bulk, is purely chemical. Chemical knowledge will teach the gardener and farmer what particular soil is best adapted for particular seed; it will teach the way of forming soils for foreign plants; of making manures to the greatest advantage; of preserving grains, roots, etc.; of destroying the insects; and of correcting the disorders injuring the valuable shrubs.

In 1810 Gerard Troost, a Dutch physician, emigrated to this country. He not only conducted what appear to be the earliest studies of the composition of Pennsylvania soils, but also made valuable contributions to agriculture, mineralogy, and geology in Tennessee.

In 1842 Samuel Luther Dana published his *Muck Manual*, which exerted a tremendous influence on New England agriculture for the next twenty years. The period 1840 to 1850 was marked by a considerable increase in scientific and industrial activities. Chemists were busy studying the chemistry of soils, crops, foods, and fertilizers.

Our brief description of prominent agricultural chemists will not be complete without the mention of Evan Pugh, the first president of The Pennsylvania State University. His well-known treatise entitled "Sources of the Nitrogen of Vegetation" established him as a leader among the agricultural scientists of his time. Although Dr. Pugh's researches were not numerous, they were characterized by an unusually high quality of skill and accuracy. His administrative record at The Pennsylvania Agricultural College was no less brilliant, and the organization of the college curriculum, the inauguration of research, and the high standards set by Dr. Pugh at that early date will always stand as monuments to his memory.

Samuel W. Johnson of Yale undoubtedly exerted a greater influence than any other agricultural chemist of his time. Although Johnson achieved a reputation as a research worker, he is best known for his ability as an organizer, administrator, and writer. He is considered one of the most capable critics of agricultural chemical literature of all time. Johnson is known as the founder of chemical regulatory work in America, owing to his inauguration of fertilizer control in the state of Connecticut.

If space permitted, we should like to discuss invaluable contributions to American agriculture made by outstanding contemporary agricultural chemists. Among others should be mentioned the work of E. W. Hilgard, Cyril Hopkins, and others in soil chemistry; of S. M. Babcock in dairy chemistry and animal nutrition; of C. A. Goessmann and Whitman H. Jordan in animal nutrition, fertilizer studies, and research administration; of L. L. Van Slyke in dairy chemistry; of Henry Prentiss Armsby in animal nutrition; and of William Frear in soil chemistry and pure food work.

In the United States no greater encouragement has been offered to agricultural science than the grants made at different times by the federal government. The Morrill Act in 1862 established our agricultural colleges; the Hatch Act in 1887 founded our agricultural experiment stations; and the Adams Act in 1906 provided means for special investigations which have meant much for the promotion of agricultural research. Passage of the Purnell Act (1925) and the Bankhead-Jones Act (1935), which provides additional funds for the prosecution of scientific research in the various state agricultural experiment stations, has served to stimulate many phases of scientific activity as well as the creation of many new research projects. In 1946 Congress passed the Research and Marketing Act, which provides additional funds to investigate "basic laws and principles relating to agriculture and to improve and facilitate the marketing and distribution of agricultural products."

Another federal agency of inestimable value to American agriculture is the Department of Agriculture at Washington, D.C. With its many bureaus and its excellent scientific personnel, not only has it played a most important role in scientific agricultural development, but also its researches have helped to better living conditions in every walk of life in every state of the union.

The establishment in 1938 of Regional Research Laboratories for the study of special problems relating to agriculture has provided even greater governmental assistance in the solution of difficult problems peculiar to the various regions of the country.

BEGINNINGS OF PHYSIOLOGICAL CHEMISTRY

Physiological chemistry or animal chemistry is an outgrowth of animal physiology. Experimental physiology was recognized as an independent division of science as early as 1800. To be sure, William Harvey had announced his discoveries regarding blood circulation as early as 1628. Similar progress had been made in the study of respiration. Robert Boyle had studied the effect of low and high air pressure on animals as early as 1659. Other important information regarding respiration was added by Robert Hooke (1667), John Mayow (1668), John Priestley (1774–1777), and Antoine-Laurent Lavoisier (1777). However, it was not until the latter part of the nineteenth century that experimental physiology really became recognized as a separate division of science.

The same trends were characteristic of the research on digestion and metabolism. Sanctorius of Padua (1614) had called attention to "insensible perspiration" and gain and loss of body weight as affected by food intake. Regnier de Graaf (1664) had described the properties of pancreatic juice, and Spallanzani (1782) the effect of saliva and gastric juice on foods. No important chemical techniques were advocated, although William Prout (1785–1850) identified hydrochloric acid in gastric juice. Prout is referred to by many writers as the first English physiological chemist. William Beaumont, an American physician, published his classical work on gastric digestion in 1833. During the same period (1600–1850) similar progress had been made in many other phases of experimental physiology.

However, it was not until the period 1850 to 1880 that experimental physiology and physiological chemistry really achieved some degree of recognition. Even as late as 1800 to 1900 there seemed to be no great appreciation of the value of the application of chemistry to physiology. Chittenden, in his *Development of Physiological Chemistry in the United States,* credits Michael Foster with the establishment of the first practical instruction of physiology in England in 1874.

Henry P. Bowditch, who had studied under Carl Ludwig in Leipzig, returned to the United States in 1871 and accepted the chair of physiology at Harvard University. Since at that time laboratory work was not considered essential, he had few facilities for experimental work. Nevertheless, with a few pieces of apparatus he had brought from Germany he set up a small laboratory in the attic of the medical building. This was the first physiological laboratory for students in the United States.

For the reasons just described physiological chemistry was slow in developing. It was a borderline science, claimed by chemistry on the one hand and by physiology on the other. Even today departments of physiological chemistry may be found which are administered by other divisions, such as medicine, biology, and physiology.

The period 1870 to 1880 was marked by the brilliant researches of French and German physiologists. Claude Bernard of Paris showed great versatility in his research; his contributions included the discovery of liver glycogen and its relation to blood sugar in health and disease, the digestive properties of pancreatic juice, and studies in muscle and nerve physiology. He was even more outstanding as a teacher and creator of research techniques. During this period Germany was a mecca for foreign students seeking advanced training in physiological and chemical sciences. Some of the most important men of this productive era included DuBois-Reymond (Berlin), Baumann (Berlin), Heidenhain and Röhmann (Breslau), Pflüger (Bonn), Pettenkofer and Voit (Munich), Hoppe-Seyler (Strassburg), Hüfner (Tubingen), and Kühne (Heidelberg). From these laboratories came a host of young workers who were to carry on the future development of physiology and physiological chemistry.

Up to this point we have emphasized the factors affecting the development of physiological chemistry in Europe. It was natural that early American physiological chemists should look to Europe for inspiration and ideas.

Russell H. Chittenden was trained at Yale University, where the first well-organized course in physiological chemistry for medical students had been established in 1874. In 1878 Chittenden went to Heidelberg, where he studied with Kühne, who had been trained in the laboratories of Virchow (Berlin), Ludwig (Leipzig), and Bernard (Paris). As a result of Chittenden's training it was but natural that Yale University should become the center of physiological chemistry in America. Chittenden was an enthusiastic and inspiring teacher and research worker. It was not long before students were flocking to his laboratory from this and other countries. As a result, most of the biochemical laboratories in this country are staffed with men and women who can trace their biochemical lineage to Chittenden.

At first, research papers on biological and physiological chemistry were published wherever editors would accept them. The *American Chemical Journal,* which was the forerunner of the *Journal of the*

American Chemical Society, was established by Ira Remsen in 1879. This journal accepted articles in all fields, including agricultural and physiological chemistry. Gradually, specialized scientific groups organized their own journals in particular fields. The literature grew rapidly, and abstract journals were created to assist the busy teacher and research worker in keeping abreast of the world literature. As a result American biochemistry grew and flourished until today this country stands second to none in this important field of science.

NATURE OF BIOCHEMISTRY

Biochemistry has been defined as "the chemistry of living things." As a result, much of the chemistry in this book will have to do with the chemistry of life processes.

"Life" is one of the most difficult words to define, owing to the fact that no one really comprehends the meaning of "the living thing." However, in an attempt to approach an understanding of life we generally classify all things with which we are familiar into two groups, the living and the non-living or lifeless. Then we proceed to justify the division by listing a number of properties, all of which are common to the living group, but some of which are uncommon to the non-living group.

One such property possessed by all living things is autonomic movement. To be sure, plants do not move from place to place, like animals, but certain plant organs are capable of movement to a limited extent. Moreover, their growth is a slow movement, and considerable movement takes place on an inter- and intracellular level.

A second property, growth, is characterized by synthetic development from within which is different from the type of growth we see in inanimate crystals which grow by additions from the outside. A third property of living matter is the power to respire. During respiration living tissues undergo characteristic oxidative changes and liberate energy. All living organisms possess the power of irritability; that is, they respond and whenever possible adapt themselves to environmental stimuli. Finally, and possibly most important, living organisms all possess a definite life cycle and have the power of reproduction. If it were not for the power of reproduction, all living matter would cease to exist.

All the above-mentioned properties of living matter depend on controlled chemical reactions. These controlled reactions, in turn, depend on the unique ability of living cells to produce essential controlling agents, called enzymes.

The Cell

The science of biology teaches us that the cell is the biological unit of life. Chemistry and physiology have shown that these tiny units may be likened to factories, each receiving its raw materials from which it not only fabricates its own building materials but, in addition, often furnishes essential materials for other cells elsewhere in the living organism.

Animal cells are covered by a protoplasmic membrane, whereas plant cells usually possess two membranes, one consisting of modified protoplasm similar to that of the cell itself, and a second membrane or heavy wall consisting of cellulose.

The living plant cell is, in reality, a protoplast, that is, a droplet of organized protoplasm enclosed in a cellulose compartment. When such a cell is plasmolyzed by immersion in a salt solution of higher concentration than that of the cell itself, the cell loses its characteristic turgidity and the protoplasmic membrane draws away from the cellulose covering.

The typical cell contains a spherical or semispherical body called a nucleus. If the nucleus is removed, the cell loses its power of reproduction. Certain cells have several nuclei, and a few are non-nucleated, for example, the red blood cells in man.

Although cells of plants and animals differ in many respects, we find that both types of cells contain, as their principal structural substance, a liquid or semiliquid form of matter known as protoplasm. Careful chemical studies of plant and animal protoplasm indicate that both types are essentially similar in their chemical and physical characteristics.

Protoplasm

Protoplasm is a translucent, grayish, shiny substance similar to a thin jelly in consistency. To use another example, protoplasm has properties similar to those of fresh egg white. Under the microscope it appears to consist of a thick matrix containing globules and granules. Chemically, protoplasm seems to be a colloidal system consisting of water (about 75 per cent) and solid matter (about 25 per cent). Protein is a major solid constituent. Other chemical constituents of protoplasm include sugars, lipides, amino acids, and inorganic salts.

At first glance it would appear that protoplasm is a rather simple system. However, it is very complex when considered from the dynamic standpoint. Thousands of diverse activities may be ascribed to protoplasm. Since protoplasm is a colloidal system (emulsion), we will learn in Chapter 2 that many of the characteristic properties of protoplasm can be best explained in terms of physics and physical chemistry. This colloidal system contains proteins, lipides, and other chemical substances which possess the unique property of attracting and holding large amounts of water within the protoplasmic structure. The presence of water gives protoplasm its characteristic jelly-like properties, causing it at times to behave as a semiliquid and at other times as a semisolid. Consequently the behavior of cell membranes, the permeability of cells, and the metabolic changes within the cells must depend, in great measure, on the condition of the protoplasm at any given moment.

The turgidity of cells depends upon interrelationships existing between water and other constituents. Consequently the uptake (imbibition) of water by cells, the viscosity of protoplasm, and the elasticity of the cell membrane require very delicate balance and control, details of which still baffle scientific workers.

Naturally a host of questions suggest themselves regarding this protoplasm: what is its function in the cell and what are the chemical activities of the cell itself; how does it receive its raw materials; what changes are produced and how are these chemical changes stimulated and controlled? Many of these questions can be answered by means of chemical research, and it is through the study of problems of this type that we are able to obtain a mental picture of the various phases of growth and development in plants and animals. However, before we can go far in the study of these questions we must ascertain, so far as possible, the chemical composition of the cell and the nature and structure of the constituents, as well as their chemical and physical properties.

Importance of Water

It is impossible to overstress the importance of water in living processes. Water is the solvent and dispersion medium for all protoplasmic constituents. Water not only acts as a transportation medium for cell nutrients throughout the living organism, but also serves as the medium in which reacting substances undergo fundamental changes. It is necessary only to cite the ascent of sap in trees and the circulation of blood in animals to emphasize the importance of water for the maintenance of life. Absorption, secretion, and excretion would not be possible without water.

From a biological standpoint it is important that water exhibits high surface tension, that it forms hydrates with many compounds, and that it possesses high specific heat. Water is a necessary reactant for the hydrolytic splitting of carbohydrates, fats, and proteins. The plant requires from 200 to 400 lb. of water to produce 1 lb. of dry matter. In spite of this, the plant maintains a very accurate balance between water and other normal cell constituents. Water exists in living tissue as free water and as bound water, or water of hydration. Both forms of water are of great biological importance.

Water cannot pass freely in and out of living cells because its passage is controlled by osmosis, hydration of colloids, and other factors. In certain diseases of man the permeability of cell membranes is altered, and normal water balance is upset. An example is the kidney disease known as nephritis. In this disease, tissues swell to abnormal size owing to excessive water uptake. The condition is commonly known as "dropsy." Diabetes is associated with dehydration and acidity. Excessive losses of water by sweating deplete the body tissues of essential mineral salts to the point where salt depletion may cause prostration.

As tissues age they lose their normal water-holding ability. As a result tissues of young organisms are richer in water than those of aged organisms. Mammals, such as dogs and man, can survive for a month or more without food if water is available. However, death will occur in but a few days if the body is deprived of water.

When deuterium (heavy hydrogen) unites with oxygen, heavy water is formed. Oxygen also exists in isotopic forms with atomic weights of 16, 17, and 18, respectively. Theoretically the two isotopic forms of hydrogen and the three isotopic forms of oxygen are capable of uniting to form 18 different kinds of water. Heavy water and isotopic forms of hydrogen and oxygen serve as tools for the biologist and biological chemist in their studies of cell metabolism.

Inorganic Salts

Mineral elements occur in living tissues as inorganic salts, as salts of organic acids, and in combination with organic compounds. Although sulfates, chlorides, phosphates, and carbonates of sodium, potassium, calcium, and magnesium are usually considered most important, salts of trace elements are often present in significant amounts. Examples of such micronutrient elements are boron, copper, cobalt, manganese, iodine, and zinc.

Phosphorus and sulfur are unique in that they form vital combinations with organic compounds. For example, certain coenzyme systems, essential for normal metabolism, contain phosphates as part of the coenzyme molecule. Certain essential amino acids contain sulfur in highly reactive form. These and other inorganic elements will be discussed in subsequent chapters.

Proteins, lipides, and carbohydrates contain carbon, combined in various combinations with other elements, of which hydrogen, oxygen, nitrogen, and sulfur are most important. These compounds play a major role in normal plant and animal growth. In subsequent chapters we shall consider the chemical structure and properties of carbohydrates, lipides, and proteins in considerable detail.

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Properties of matter

2

The regulation of physiological processes involves an impressive number of physical variables. Hence the study of protoplasm depends extensively upon the physical sciences. Certain aspects of these sciences fundamental to an understanding of biochemistry are assembled in this chapter. The concept of the state of matter and various properties of biological importance when matter exists in one or more of these states are included in the discussion.

GASES

Physical Behavior

In gases the individual particles are relatively far apart and move at high velocities in a rather random manner. The molecules exert little attraction for one another and possess energy of motion sufficient to overcome this attraction. Thus the particles distribute themselves throughout any evacuated space into which the gas is placed. As a result the gas assumes the volume and shape of the system. The gas has no volume or shape inherently its own.

The molecules travel straight paths except for occasional collisions with one another, but even after colliding the motion is linear, the molecules merely being deflected at some angle with respect to the former path. When a molecule collides with a wall bounding the gas, the molecule changes direction. In so doing a force is exerted on the wall, and the effect of repeated collisions with a given wall area is called pressure. Thus a confined gas exerts a pressure on the container that depends upon the velocities of the molecules, their sizes, and their number.

Studies of gases have disclosed quantitative relationships in their behavior. For example, if the temperature of a given sample of gas is kept constant, an application of external pressure compresses the gas and its volume decreases. On the other hand, when the volume of this sample is kept constant and the temperature is raised, the pressure exerted by the gas increases. It is known that molecules travel faster as the temperature increases. Hence they collide more often with the walls of the container and exert an increased pressure. Furthermore, if the number of gas particles is increased by increasing the size of the sample, the number of collisions per unit area and the pressure are increased. These effects can be written mathematically as proportionalities:

$$P \propto \frac{1}{V} \qquad (\text{constant } n, T)$$

where P = pressure, V = volume, T = absolute temperature, and n = number of moles of gas = weight divided by molecular weight. And

$$P \propto T \qquad (\text{constant } n, V)$$
$$P \propto n \qquad (\text{constant } V, T)$$

These expressions may be combined to

$$P \propto \frac{nT}{V}$$

and incorporation of a proportionality constant R called the gas constant yields the ideal gas law

$$P = \frac{RnT}{V} \quad \text{or} \quad PV = nRT$$

The constant R possesses the same value for all gases or mixtures of gases and once evaluated may be used in all calculations with the ideal gas law. Indeed this same constant appears in many other expressions employed with other types of systems.

It is customary to use the word "ideal" in connection with this law because common gases do not obey it exactly. In fact the deviations can be quite striking. Nevertheless, the ideal gas law is useful and proves satisfactory for most purposes when (*a*) the pressure is relatively low and (*b*) the temperature is rather far above the boiling (liquefying) point of the gas involved. Thus the ideal gas law is readily applied to gases that boil far below room temperature and to other gases or even to materials liquid at room temperature if the actual temperature of the system is sufficiently above the boiling point. For other cases special equations have been developed, but these are more complex and even they cannot be accurately applied to all gaseous systems. Hence the ideal gas law is by far the most widely applied expression.

Errors in the use of the gas law appear to arise from attractions between the individual molecules and from the volumes of the molecules themselves. Thus the errors diminish when the molecules are far apart. In this case the fraction of the total volume occupied by the molecules themselves is very small. In addition, the farther apart the particles, the less they affect one another. Hence a decrease in pressure improves the accuracy. Recall that increasing temperature increases molecular velocity, which in turn helps overcome the forces of attraction between molecules. This effect likewise improves the application of the ideal gas law.

Transport of Gases in Nature

Living cells utilize various gases in their physiological processes. Depending upon the cell and the biological reaction concerned, a gas may be consumed or given off by a cell. Hence cells depend in part for their livelihoods on direct or indirect contact with gaseous systems. Therefore, some sort of gas transport is required, the nature of the mechanism depending on the gas and the requirements and location of the cell.

Certain cells have a portion of their external surfaces in contact with a gas phase, frequently air. In this simplest situation gases are exchanged through the cell wall by diffusion, a process depending upon the random motion of molecules. Diffusion may be illustrated by the distribution rapidly occurring when a sample of gas is introduced into a system. Samples taken from all parts of the system reveal the presence of the newly introduced gas. Even though another gas be already present, the new sample distributes itself almost as though the system was originally empty.

When a porous barrier is interposed, gas molecules diffuse through the pores to reach and fill that portion of the system on the other side of the barrier. As soon as some molecules pass through the barrier, their random movements cause a few to return. However, the number moving through the barrier in a given direction is proportional to the number of molecules on the side from which the movement took place. Thus, with a higher concentration on one side of the porous barrier, a net transfer of gas takes place and continues until concentrations become equal on both sides. A cell using oxygen, for example, keeps the concentration of oxygen low in its interior by consuming oxygen molecules in one or more reactions. Then additional oxygen diffuses in from the outside as long as the external concentration exceeds the internal concentration.

This diffusion process transfers gases in all biological systems. Gases diffuse through porous barriers, from one part of a solution to another, and through solid barriers in which the gases dissolve. In this last case a gas reaches the barrier by diffusion, some molecules strike it and dissolve, a part of these diffuse in solution to the other surface of the barrier, and some diffuse away into the adjoining system. As long as the concentrations differ on the different sides of the barrier, more gas passes in one direction than the other and there is a net transfer. In actual cells it is common for more than one gas to be transferred or exchanged. Since gases move independently of one another, different ones may be diffusing simultaneously in the same or in different directions.

Many cells are not in contact with a gaseous system. Part of these are immersed in soil solutions, liquid cultures, natural bodies of water, and the like, which are in turn in contact with a gas phase. In this situation gases are exchanged by diffusion between gas and liquid phases and between liquid phases and the cells. Here more stages are involved in the overall gas exchange, and the complete process takes somewhat longer. Yet it is efficient enough for many organisms, including most of the small forms.

To promote more rapid gas transfer, diffusion has been supplemented, especially in the larger organisms, by auxiliary mechanisms. One of these is protoplasmic streaming, common among the larger single-celled species and among cells of multicellular organisms. The protoplasm of these cells consists partially of a viscous liquid in which currents produce a circulation that supplements diffusion in the transfer of gases and of other dissolved materials. Outside the cells there are specialized circulatory systems in the higher plants and animals. Such systems are specifically adapted in part to aid in the transport of gases in either gaseous or solution states. In the former case, pores provide air passages among the tissues, bringing more cells into contact with the gas phase. This development is elaborated still further when volume control is provided in respiratory chambers, pumping gases in and out and thus bringing them into contact with specialized cells. Gases in solution are circulated by means of flowing blood and lymph in animals and by the movement of solutions through plants. The various circulatory systems are considered in more detail in later chapters.

Biological Functions

The principal biological functions of gases fall into two categories. Gases are required in the utilization of foodstuffs and might thus be considered as foods themselves, or they are the by-products of food utilization and biological processes in general. Different species take up different gases, including oxygen, carbon dioxide, hydrogen, nitrogen, hydrogen sulfide, and methane. Gases eliminated include carbon dioxide, oxygen, nitrogen, hydrogen, and methane—in other words, many of the same gases. In certain cases a given individual may consume one gas, while under different conditions the same gas may be given off and another taken up.

In another somewhat incidental but important function, carbon dioxide and ammonia dissolve in biological fluids to form buffers, valuable in controlling the acidity of those fluids. Gases are employed by swimming and floating species in providing the buoyancy necessary to prevent sinking and resultant immobility. Finally, air is a medium of movement possessing sufficient substance for the flight of insects, birds, and bats.

LIQUIDS

In the liquid state the particles are in continuous motion and move rather freely throughout the liquid. However, the particles are much closer together than those in a gas, and there is much more interaction between them. Freedom of motion is nevertheless great enough to permit relative movement of layers of liquid. Thus liquids possess the ability to flow and to take the shape of the container. On the other hand, liquids do not fill containers regardless of size, as do gases, because the individual molecules seldom leave the neighborhood of their fellows and escape from the liquid. At a given temperature, the molecules of a liquid maintain a given average distance from adjacent molecules regardless of the size of the containing system. Nor is this spacing very much affected by pressure. Even temperature variations produce but slight and gradual changes until the liquid boils or freezes.

Vapor Pressure and Boiling

In any substance the molecules are in motion, and they possess an average velocity characteristic of the temperature. An increase in

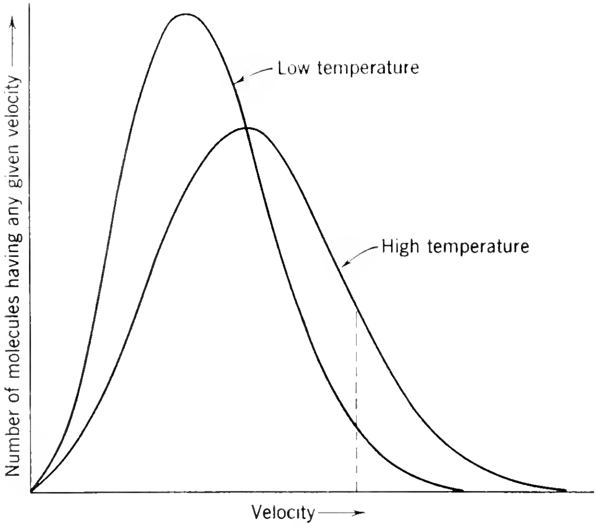

FIGURE 2–1. The velocities of molecules in a gas. An increase in temperature increases the average velocity by increasing the numbers of molecules having the higher velocities. If the dashed line represents the threshold velocity required before a molecule can undergo a process, a much larger fraction of the molecules present can participate in that process when the temperature is raised. The area under a curve is proportional to the total number of molecules. Both curves delimit equal areas when the amount of gas is kept constant. The areas under the curves and to the right of the dashed line are proportional to the numbers of molecules sufficiently energetic for the process.

temperature increases the average velocity. However, in no case do all the molecules in a material possess the same velocity. Rather there is a relatively broad range of velocities with some moving slowly, some rapidly, and many at intermediate speeds. The typical distribution curve of the molecular velocities is shown in Figure 2–1.

The strong intermolecular forces in liquids tend to limit the paths of individual molecules to the body of the liquid. Whenever a molecule reaches the liquid surface, the field of force surrounding it is unsymmetrical. On the liquid side of the surface there is a strong attraction restraining escape of the molecule. From beyond the surface there is little attraction. Therefore, liquid molecules do not escape from the surface into the gas phase above unless they have sufficient energy or velocity to break away from the restraining attraction of their fellow liquid molecules. Ordinarily some molecules do escape by virtue of their high velocities. This process is called evaporation. The rate of evaporation is dependent on the fraction of molecules possessing velocities greater than the critical value allowing their escape.

If a liquid is placed in a closed container, molecules evaporate into the gas phase and move randomly about. A portion of these escaped molecules wander back, strike the liquid surface, and stick to it. When the rates of escape and return are equal, the system is said to be in equilibrium, and net change ceases. At equilibrium there is a constant number of molecules per unit volume of gas phase, depending upon both the liquid and the temperature. This constant number of molecules exerts a pressure determined, according to the gas law, by the quantity of gas. In systems of this type the term *vapor pressure* is used and defined as the pressure exerted by a vapor in equilibrium with the corresponding liquid.

Since average velocity increases with temperature, vapor pressure increases also, but the relationship is not linear. See Figure 2–2. Vapor

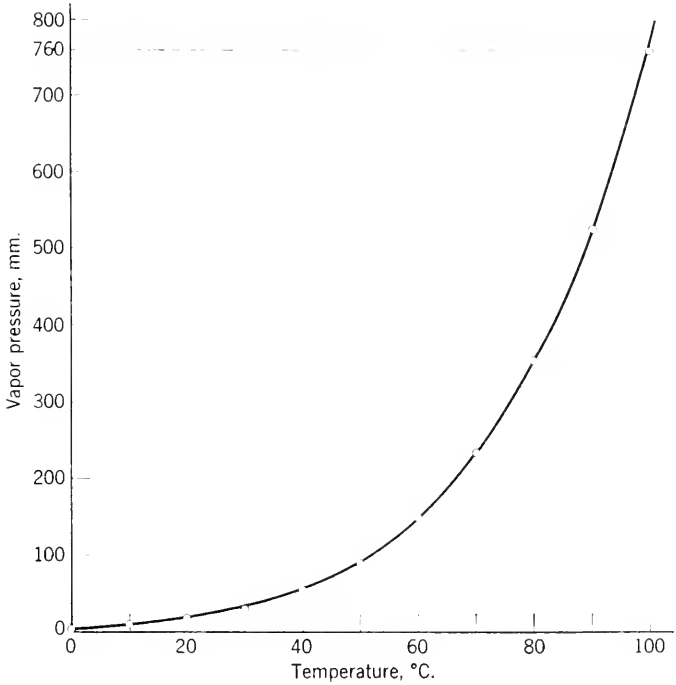

FIGURE 2–2. The vapor-pressure curve for water. Other liquids possess very similar curves displaced along the abscissa and intersecting the 760-mm. line at different temperatures corresponding to their particular boiling points.

pressure increases more rapidly than temperature, yielding the observed curve. Figure 2–1 accounts for the curvature. In a typical situation, a temperature rise shifts the entire curve for the distribution of velocities. Since this plot is itself a curve, a small temperature change greatly affects the fraction of molecules having velocities in excess of the critical escape value. Thus the vapor pressure increases as this fraction, and a curve like that of Figure 2–2 results.

When the temperature is increased until the vapor pressure just equals the external pressure, bubbles of vapor form in and escape from the body of the liquid. This temperature is called the boiling point. Clearly the boiling point depends upon the external pressure, which must be matched by the vapor pressure. In the example of Figure 2–2, the vapor pressure of water equals the normal atmospheric pressure at 100°C., and water boils at this temperature under standard sea level pressure conditions. If the pressure is reduced by ascending to a high altitude or by means of a vacuum pump, the boiling point is correspondingly reduced.

Liquids are often boiled and the vapor then brought into contact with a surface cooled below the boiling point. The vapor molecules are thus cooled, slowed down, and condensed to form liquid again. This process is called distillation and is widely employed in separating materials having different boiling points. Artificial reduction of the external pressure, followed by boiling and subsequent vapor condensation, is called vacuum distillation. This process is widely employed in separations at low temperature when the boiling temperatures at atmospheric pressure are high enough to cause decomposition of desired materials. For example, the distillation of water at 100°C. in the manufacture of condensed milk leads to undesirable changes in flavor and nutritional components in the product. When the distillation occurs at low pressure and therefore at low temperature, a much more acceptable condensed milk is obtained.

When two liquids are mixed, each exhibits a vapor pressure depending upon the natures of the individual liquids, their relative quantities, the temperature, and the attractive forces between the molecules of the two liquids. This last factor is important and produces striking effects. When the unlike molecules attract each other very strongly, their escaping tendencies are lower than expected. When these attractive forces are unusually low, the vapor pressures are higher than anticipated. Such liquid systems boil when the combined vapor pressures equal the external pressure. Thus the anomalous intermolecular forces lead first to anomalous vapor pressures and finally to anomalous boiling points.

When a non-volatile material, one of low vapor pressure, is dissolved in an ordinary liquid, the vapor pressure of the resulting system is due essentially to the liquid alone. Since the concentration of the liquid in the solution is reduced when compared to the pure state, the vapor pressure of the solution is also reduced. Hence the vapor pressure of blood is due to the water present but is less than that of pure water at the same temperature because in blood the water molecules are diluted by all the other materials present.

Surface Tension

The non-uniformity of the force field acting on the molecules in the surface of a liquid has been discussed in the preceding section. This lack of symmetry leaves the surface molecules with forces on the surface side that can be satisfied only as extra attractions between neighboring molecules in the surface. Such extra attractions draw the molecules somewhat closer together, increasing the density of the surface layer. As a consequence the surface layer behaves like a film and is capable of supporting small objects of greater density than the liquid itself, provided these materials are not wet by the liquid. Thus a greasy needle or razor blade or certain insects float on water.

The extra force between neighboring surface molecules is called surface tension. It leads to the film-like character of the surface of a liquid. Moreover, it represents a tendency for the surface to contract to the minimum area compatible with other forces acting on the material. Hence small droplets are spherical, this form having the lowest possible area to volume ratio. Drops of larger mass are subject to correspondingly greater gravitational force and when placed on an unwetted solid are flattened more or less, depending whether they are large or small.

When a liquid contacts a porous solid that it does not wet, surface tension minimizes the area of liquid surface as usual and prevents entrance of liquid into the pores. This type of behavior is encouraged by waterproofing textiles to keep water from entering small spaces but is objectionable when attempting to pass water into and through certain thoroughly dried soils. In both cases the water wets the solid with difficulty or not at all and is excluded from pores and cavities.

On the other hand, a liquid that wets a solid is drawn into small openings in the solid. In this situation the solid behaves as though it is completely covered by a very thin liquid film. The liquid-gas surface is then minimized by movement of liquid in the small capillary spaces. These openings may be completely filled or not, depending on the balance struck between the opposing tendencies of surface tension and gravity. Thus a porous solid whose surface is wet by water becomes impregnated with water and may serve, like ordinary soil, as a water reservoir. Movement of liquid through pores brought about by surface forces is known as capillary rise or transfer and is generally believed to play a role in the movement of biological fluids, especially in plants and soils.

Determination of surface tension is of value since the magnitude of the various surface phenomena depends in turn upon the magnitude of the surface tension. A number of measuring methods are available, all providing comparable results. These methods depend upon the height a liquid reaches in a capillary of known diameter, the size to which drops of liquid grow before breaking away from a tip of fixed dimensions, or the force required to tear a standard ring from the liquid surface. All these principles are widely used, and the various equations are available in any textbook on physical chemistry.

Viscosity

Liquids are also characterized by their resistance to flow. This resistance or internal friction is known as viscosity and has its origin in the attractions between molecules. As a result it takes force to move one layer of liquid past another, the magnitude of the force being a measure of the resistance offered to flow. This force can be applied in gravitational form, by means of gas pressure, or as a torque from a rotating metal cylinder. Viscosity then equals the force applied times the interval of time divided by the area of the shearing or flowing surface. Since the latter usually cannot be readily measured, it is customary to relate the time of flow of one liquid to another in the same apparatus. If the absolute viscosity of the reference liquid is known, the value for the first liquid is readily calculated. Methods and calculations are described in elementary textbooks on physical chemistry.

Each liquid system possesses a characteristic viscosity depending upon the sizes and the interactions of the particles present. Large molecules and large intermolecular forces lead to high viscosities. Likewise temperature has an effect, probably involving velocity effects like those discussed on page 21 under vapor pressure and boiling. At any rate, an increase in temperature commonly decreases viscosity, presumably because the increase in molecular velocity helps overcome intermolecular forces.

The foregoing considerations apply in general to solutions in the liquid state as well as to pure liquid. If the dissolved materials contain relatively large molecules, they enhance the viscosity of the solution compared to that of the solvent. On the other hand, if the dissolved molecules are moderately small and interact but weakly with each other and with the molecules of the liquid, viscosity is reduced. Such is the case with solutions of ethyl alcohol or oxygen in water, for example. In any given kind of solution the viscosity will be related to concentration, but it is not yet possible to predict viscosity from theoretical considerations alone. Hence concentration can be determined from viscosity only by comparison with an experimental standard series.

The Liquid State in Nature

Pure liquids are non-existent in nature, but solutions of the liquid type are very widespread. Even rain drops, about as close a natural approach to a pure liquid as there is, are solutions of atmospheric gases in water. Liquid solutions, mainly aqueous, are fundamental to biology. The environments of most cells are liquid since many species are suspended in solutions and many others are bathed in liquids circulating and diffusing about in the interiors of multicellular forms.

Liquids possess certain attributes of special biological importance. They are dense enough to provide support against gravitational forces. Yet liquids have viscosities low enough to allow the movement of motile forms. Thus liquid provides an especially suitable environment for life, so much so that life on earth is presumed to have originated in a liquid system.

Biological Role of Water

Oxygen is the most abundant element of the earth's crust. Hydrogen, though much less abundant on a weight basis, is also exceedingly plentiful on the basis of the number of equivalents, owing to the low atomic weight of this element. Although both oxygen and hydrogen are distributed among a great many kinds of compounds, together they make up water, one of the most common substances of the earth's crust. In fact water is so abundant and so widely distributed that it is universally available on the surface of the earth. It is the only liquid naturally occurring in such enormous quantity, including even petroleum. Moreover, the characteristic physical properties of water favor a very wide distribution. The freezing and boiling points are such that the normal temperature fluctuations of the earth lead to vaporization of water, wide distribution of the vapor, and condensation over the surface leading to rain or snow everywhere.

This ubiquitous nature of water, especially in liquid form, suits it admirably for a major biological role. Indeed it is difficult to imagine the origin of life as we know it without an abundance of water. Even though another liquid might be proposed as a replacement, the unusual properties of water make it a unique basis for the life of the earth.

A complete understanding of the unusual properties of water is not yet available. Yet some ideas have been proposed to account for the behavior of this strange substance. As usually represented by chemists, water has the formula H_2O, corresponding to a formula weight of 18. However, this low value would indicate a low boiling point for the substance since there is a correlation between molecular weight and boiling point in a series of similar compounds. This comparison of water in Table 2-1 with the members of two of its chemically related families illustrates its unique position. The boiling point of water compared to the boiling points of the other members of the inorganic group at the left suggests that perhaps the individual water units are associated in the liquid state. Otherwise water ought to boil below −60°C., perhaps about −65°C. If association gives rise to the high boiling point, extrapolation indicates that the aggregates of water might have average relative weights of about 200.

TABLE 2–1. The Boiling Points of Water and Certain Related Compounds

Substance	Formula Weight	Boiling Point, °C.	Substance	Formula Weight	Boiling Point, °C.
H_2O	18	100	Water	18	100
H_2S	34.1	−59.6	Methanol	32	64.7
H_2Se	81.2	−42	Ethanol	46.1	78.4
H_2Te	129.6	−1.8	*n*-Propanol	60.1	97.8
			n-Butanol	74.1	117
			Cetyl alcohol	242.7	344

When compared with the alcohols the position of water is not quite so extreme. Yet water is anomalous even here. Moreover, comparison of the alcohols with the inorganic substances suggests that even the alcohols may be somewhat associated in the liquid state. Unfortunately no direct determination of molecular weight in pure liquids has been devised, so these speculations have not been verified. Still there are a few experimental indications that liquid water consists of aggregates of subunits. These aggregates are presumed to dissociate rapidly and reversibly, leading to a dynamic system containing structures of various sizes.

Existence of aggregates in water implies the presence of strong intermolecular forces of attraction binding the subunits together. These forces are believed to be electrical in character and arise from the unsymmetrical structure of the individual molecule. The three atoms of water do not lie along a line. Rather they form an angle of 105° with the oxygen atom at the point of the angle. In addition to this effect, oxygen is relatively more electronegative than hydrogen; that is, the electrons of the bond between hydrogen and oxygen are displaced slightly toward the latter. In turn this shift causes a slight imbalance in the electrical charge on the molecule. Thus the oxygen is slightly negative and the hydrogen atoms relatively positive. Such separation causes the molecule to act like a miniature magnet, and, like magnets, molecules of this type attract each other and associate as suggested for water. In Figure 2–3 ⊕ signifies a very small positive charge, ⊖ a small negative charge of twice the size, and the dotted lines suggest the interactions leading to aggregation. These attractions, termed hydrogen bonding, are weak compared to the ordinary bonds between hydrogen and oxygen and break and re-form rapidly and continuously in the liquid.

The slight separation of charges characteristic of certain molecules is called a dipole, and these molecules are said to be polar. Whenever dipoles exist they attract one another even though the molecules

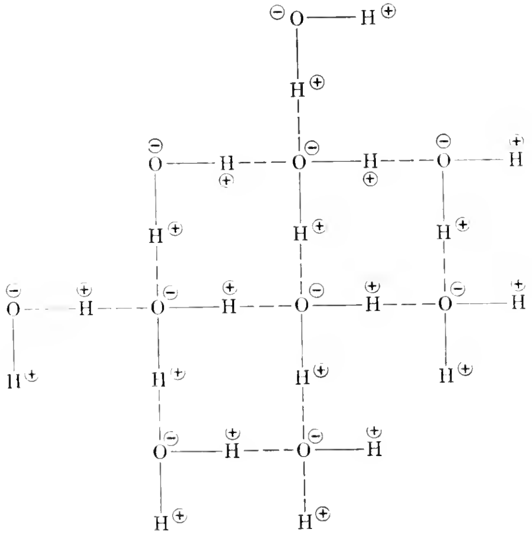

FIGURE 2–3. A schematic representation of the structure of water. Presumably the lattice is three dimensional instead of two as drawn and may extend to an unknown degree in any direction.

themselves may be of different kinds. Thus polar molecules interact and polar substances tend to be most soluble in polar liquids. It so happens that a great many molecules of biological importance are polar and thus are soluble in water. Indeed water is the solvent *par excellence* in biology, thus fulfilling yet another vital role.

Water shows unusual aspects in almost all its physical properties, and all these features are biologically important. For instance, water has an exceptionally high specific heat, thus exerting a moderating effect on temperature changes. Climatological stabilization by the proximity of large bodies of water may be cited as a well-known illustration.

Everyone knows that ice floats in water. Yet a density in the solid state below that for the liquid is rather a rarity. Conceive of the consequences if water were an ordinary substance in this respect. Exposure of water to cold air would produce ice at the surface, but this solid would sink and accumulate until the liquid was completely frozen. Even oceans would freeze solid when deprived of the insulating effect of a layer of floating ice. Melting of the surface would occur in warm seasons, but huge quantities of ice would persist in deep water. Thus life could hardly become particularly abundant in the oceans. Moreover, the land might become much more arid than now without open water throughout part of the year to recharge the atmosphere with moisture.

In addition to these and other important physical roles played by water, this substance is of particular chemical consequence as well. As will be illustrated later, water is often either consumed as a reactant or appears as a product in the metabolic reactions of living things. Thus water is a critical material in both photosynthesis and respiration.

The dipolar character of water molecules leads to their association with other charged particles, a process called hydration. In this way some of the water of cells is restricted in its movement, at least to a limited and temporary extent. Some investigators have felt that water so involved is tightly and rather permanently attached and have called it "bound water." Moreover, they have attempted to show experimentally that much of the water in cells may be in this state. However, other types of experiments yield results in disagreement with such an interpretation. The basis and application of these latter findings seem sound, and rather serious objections have been made to the earlier work. Hence, most current opinion holds that water of hydration is in rapid equilibrium with the ordinary liquid water of cells. Furthermore, it is believed that water of hydration either is a small fraction of the total water or its solvent properties are not much affected by the dynamic association. Hence, "bound water" may not be as important as was once thought in explaining the winter hardiness of certain plants or water retention by others under dry conditions.

SOLIDS

This third state of matter is represented by those rigid materials whose subunits are ions or molecules. These subunits are characteristically organized into fixed, regular arrangements called crystals in which motion is confined largely to movements about average fixed sites. Thus molecules (or ions) do not wander randomly but vibrate about definite locations. Hence, materials in the solid state do not flow readily and are relatively rigid, holding a position until broken.

Phase Diagrams

Even solids, however, possess a few molecules of sufficient energy to escape from the surface as vapor and give rise to a vapor pressure in the same way as that of liquids. Naturally the forces between the molecules of a solid exceed those in liquids, thus reducing the rate of escape and hence the vapor pressure. Indeed the vapor pressures of many solids are exceedingly small, and the rate of evaporation is negligible. Others do evaporate slowly, as illustrated by ice, which passes into the gas phase on exposure to dry air. This transfer without involving a liquid phase is called sublimation. It is the basis of the use of moth balls and gives rise to the odors characteristic of so many solids.

Thus substances may exist in any of the three physical states of matter, and any one state may be converted into either of the others. The effects of temperature and pressure on these transitions are shown in plots called phase diagrams. The diagram for water (Figure 2–4) is taken as an illustration, being the most important biological case. In this system of curves, two characteristic points appear. The critical point represents the upper limit of distinction between liquid and vapor, for above the temperature at this point the vapor cannot be liquefied, no matter what pressure is applied. For water this point is reached at 374°C. The triple point represents the temperature and pressure where the three states are in equilibrium with each other. For water the point is at 0.0099°C. and a pressure of 4.58 mm. of mercury. The curves themselves indicate regions of stability between two different states in contact with each other. From such a diagram it

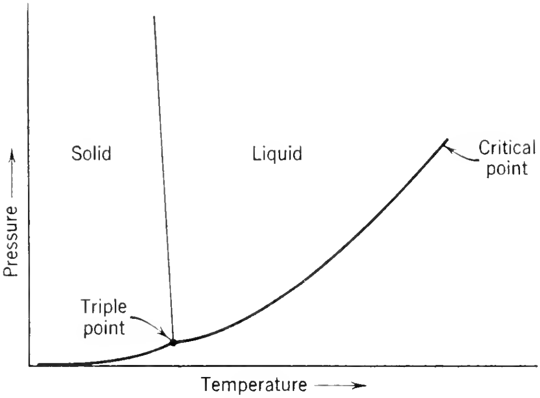

FIGURE 2–4. The phase diagram of water. The figure is drawn out of scale to reveal clearly the trends of the three individual curves.

can be predicted that water will evaporate, sublime, freeze, melt, or remain unchanged, depending upon the conditions altered.

Biological Effects of Freezing and Thawing

Since water is the principal component of living cells, the latter freeze at or just below the freezing point of water itself. Profound biological changes result from the change in state, but the reason for these changes is not always clear. Some may arise in part from the reduced rate of chemical reactions when temperature is lowered. Reactions in cells are often blocked by freezing because reactants must diffuse to a particular site before they can combine. Change from a liquid to a solid state prevents this necessary diffusion and the ensuing reactions. However, when the cells are warmed to their original temperatures, their processes should resume at normal rates if the above factors are the only ones involved. Quite often cells do not recover but are killed by the freezing and thawing. Hence other changes must be taking place.

Since water expands on freezing, it has been suggested that the increase in volume ruptures cell membranes and allows essential components to leak out when the cell thaws. Killing may occur this way but often there is no sign of cell rupture. To account for death of apparently intact cells, it was then proposed that growing ice crystals might develop between essential cell structures and force them permanently apart. But in this case also supporting evidence is usually not available, for large ice crystals can seldom be found; yet the cells are killed.

The view preferred at present introduces a new idea altogether. As cells freeze, the solid formed is essentially pure ice, while the dissolved materials become more and more concentrated as more ice forms. Finally these materials begin to come out of solution as solids, and certain of them, especially some of the proteins discussed later, change in the solid state to forms that do not redissolve on melting of the ice. These proteins are required in many cellular operations, and many must be dissolved in order to function. Hence the cells cease to operate. Perhaps this concept should be combined with all the others in explaining death by freezing, or perhaps death results from poisons that accumulate in the cells at low temperatures. Only further experimentation can answer the problem.

Some microorganisms can be repeatedly frozen and thawed and still live. Others are quite sensitive to such treatment. Multicellular animals are usually killed by freezing and so are many plants. Yet the woody plants of cold climates freeze repeatedly during a single winter and still survive. On the other hand they may be quite sensitive in the middle of their growing seasons. In recent experiments it has been possible to freeze hamsters solid and thaw them out again without serious injury. So, in addition to the causes of death by freezing, the precise effect of the treatment and the nature of the variations between species must still be explained.

SOLUTIONS

Solutions are mixed systems with one or more substances uniformly distributed in another. In addition, the distribution is commonly at the level of individual molecules or ions, rather than involving aggregates. Solutions fall into three categories based on physical state.

When two gases are mixed, they intermingle at the molecular level, forming a solution whose physical properties are typically those of gaseous systems. Thus any gases not reacting chemically form solutions that behave according to the ordinary gas laws. Hence solutions in the gas state require no special consideration.

Gases, liquids, or solids may be suitably dispersed in solids to yield solutions in the solid state. Solutions of this type may prove to be very important in biology, but they are not well understood and are not readily susceptible to quantitative description.

Finally, the dispersion of solid, liquid, or gas in liquid gives the widely studied and biologically important liquid system whose physical and chemical properties differ from those of the individual components in the system. It is customary to call the dispersion medium the solvent (usually the major component on the basis of quantity). The material dispersed is known as the solute. Occasionally when liquids are combined in equal amounts, either might be regarded as solvent or solute, and the two terms do not really differ in meaning. Certain properties of liquid solutions are of special interest in the present context and will be considered briefly in the following sections.

When dissolved in liquids, certain solutes are dispersed as charged particles called ions, and the solutions thus formed conduct electricity. As a result these materials are known as electrolytes. If solutes are dispersed as uncharged molecules, the solutions are non-conductors and the solutes are non-electrolytes.

Surface Tension

The forces leading to characteristic properties for the surfaces of liquids may be modified when solutes are dissolved. These added particles possess force fields of their own interacting with those of the solvent molecules. These interactions in the surface layers may markedly change the strength of surface films. Many electrolytes, like inorganic salts, acids, or bases, have little effect on the surface tension of water or perhaps increase it very slightly.

A number of non-electrolytes have dipole moments making them somewhat polar, and these compounds are more or less water soluble. Moreover they also lower the surface tension of water, often markedly. Common groups of substances in this category include monohydroxy alcohols, ketones, aldehydes, some esters, organic acids, and the like.

Some electrolytes possess one large ion with the charge at one end, the remainder of the ion being non-polar. Such ions tend to dissolve in water because the charges interact with the dipoles of water, but the large non-polar groups resist contact with the water. As a result, the polar portions tend to disperse in the water, the non-polar portions tend to remain out, and the ion as a whole is concentrated in the surface. In turn this concentration partially supplants the polar water molecules. In general, non-polar substances have low surface tensions so the surface tensions of aqueous solutions coated with the non-polar groups are low. Soaps, bile salts, lecithins, and household detergents function in this way and are useful because they lower the surface tension and assist in spreading aqueous systems over oily surfaces not ordinarily covered by pure water. Since many biological solutes lower the surface tension of water, biological fluids in general possess surface tensions distinctly below that of water itself. Low surface tension increases the wetting of non-polar or greasy surfaces and should aid in wetting certain kinds of cell surfaces. Wetting is necessary in turn for the transfer of solutes through these surfaces.

Certain of these materials are concentrated in the surface, producing a film of high viscosity. When gases are bubbled through such solutions a froth or foam is formed and is stabilized by the high viscosity of the film. Proteins, for example, exhibit this behavior, leading to foams like meringue. Addition of any material that markedly lowers the viscosity of the film causes the foam to break. Likewise, addition of something that replaces the solute originally concentrated in the surface breaks the foam when the new film layer has a low viscosity.

Osmosis and Osmotic Pressure

When two gases are brought into contact, they diffuse by way of molecular motion and rapidly come to equilibrium in a uniform mixture. During the diffusion process, local differences in the concentrations of the two gases produce a force responsible for uniformity of mixing. A similar phenomenon is observed when solutions of different concentration are brought together. Owing to the greater friction between molecules of solute and solvent in the liquid state, diffusion is less rapid. Eventually, however, the system comes to equilibrium and the solute is uniformly distributed throughout.

When a membrane through which solvent but not solute can diffuse (called semipermeable) is placed between solution and pure solvent, solvent diffuses into and dilutes the solution. This flow is termed osmosis, defined as the net transfer of solvent from a dilute solution through a semipermeable membrane to a more concentrated solution. Osmosis continues until a pressure develops or is applied of sufficient magnitude to exactly oppose the force of diffusion arising from the difference in concentration. This compensating pressure is called the osmotic pressure and depends upon the concentration of the solute restrained by the membrane. When the osmotic pressure is applied to the system, osmosis ceases and osmotic equilibrium is attained. However, as in other equilibria, the system is not completely static. Rather, diffusion of solvent continues, but the rates through the membrane are the same in the opposing directions. Thus there is no net transfer of solvent.

Osmotic pressures may be deliberately applied, as with piston pumps, columns of liquid, etc. Or they may develop in a closed system like a cell by a mechanical resistance to the increase in volume resulting from diffusion of solvent. Thus a cell with its solutes unable to diffuse through the cell wall swells with solvent until the strains of stretching exert the osmotic pressure and osmosis ceases. In the event that the cell wall is not sufficiently strong, the cell bursts and the semipermeable membranes are broken, allowing unimpeded diffusion of the solutes. On the other hand, a cell placed in a very concentrated solution loses solvent by osmosis and may be partially collapsed as the volume decreases. Usually this latter change does not rupture the semipermeable membrane, so a change in the external concentration will restore the normal shape.

Membranes differ in their permeability. Some retain small solutes and others allow small ones to pass through, retaining only very large molecules. Cells themselves differ in this regard with their age, temperature, nutritional status, and the presence of a variety of materials in the environment.

Membranes may actually be chosen to permit a separation of solutes. When one or more solutes are retained by a membrane and others diffuse through, the process is called dialysis. Repeated replacement of solvent permits complete diffusion and removal of all the diffusible solutes. Biological systems depend upon the dialysis of waste products out of, and the dialysis of food materials into, cells. Considerable laboratory use is also made of the phenomenon in isolating and purifying all kinds of solutes.

Dissociation of Water

Pure water exhibits a slight electrical conductivity, indicating that small numbers of ions are present. These ions must, therefore, arise by dissociation of a few of the water molecules. This process may be represented by

$$H_2O \rightarrow H^+ + OH^-$$

This proton represented by H+ does not appear to enjoy an independent existence in the presence of water molecules. Moreover, the process is reversible in that the ions recombine to form a molecule. So the overall scheme is represented more accurately as consisting of two steps:

$$\begin{aligned} H_2O &\leftrightarrows H^+ + OH^- \\ H_2O + H^+ &\leftrightarrows H_3O^+ \end{aligned}$$

which are combined to

$$2H_2O \leftrightarrows H_3O^+ + OH^-$$

This process is described quantitatively by means of an equilibrium constant which expresses the effect of the concentrations of both reactants and products. For water the constant is

$$K = \frac{[H_3O^+][OH^-]}{[H_2O]^2}$$

where the brackets denote molar concentrations of the various quantities. It turns out that $[H_2O]$ is very much greater than either $[H_3O^+]$ or $[OH^-]$ and is affected so slightly by changes in the last two that $[H_2O]$ is in effect a constant. So for simplicity one may say that a new constant $K_w = K[H_2O]^2$, and

$$K_w = [H_3O^+][OH^-]$$

Values of the dissociation constant K_w change with temperature and must be determined accordingly. There are additional effects when other electrolytes are present in relatively high concentrations. However, this latter problem is usually minor and is quite difficult to take into accurate account besides, so it is commonly neglected.

The important implications of the dissociation, or in this case ionization, constant are these. A constant fraction of the water present is ionized regardless of the amount of water involved. And the addition of either or both ions leads to enough recombination of ions to preserve the equality of K_w with the product of the two ion concentrations. Conversely, if some of one kind of ion is removed, additional water dissociates to preserve the equality. Stated in other words, if the concentration of either ion is increased, the other automatically decreases and vice versa.

At 25°C., $K_w = 1.008$ times 10^{-14}, and if $[H_3O^+]=[OH^-]$ the concentrations of both ions equal 10^{-7} molar. All aqueous solutions having equal concentrations of hydrogen (hydronium) and hydroxyl ions are said to be neutral and correspond in these concentrations to pure water. Note that both are $10^{-7}M$ at 25°C. But a change in temperature changes K_w and hence changes the values of $[H_3O^+]$ and $[OH^-]$ at neutrality. When $[H_3O^+]$ exceeds $[OH^-]$ a solution is said to be acidic, and when the reverse is true the solution is alkaline.

The dissociation reaction written for water takes no account of the aggregation of the molecules discussed earlier. The effect of this aggregation on the reaction and its constant cannot be evaluated. Fortunately, the influence is constant and introduces a factor appearing in K_w. Perhaps a part of the change of K_w with temperature reflects a change in the structure of the water aggregates. If so, it is not yet subject to experimentation.

Acids and Bases

A number of compounds are called acids or bases. When dissolved in water, the acids increase the concentration of hydrogen ion and decrease the concentration of hydroxyl ion. Bases have the opposite effect. In addition, acids and bases function in the absence of water and consequently without involving hydrogen or hydroxyl ions. Hence some more general definition than production of aqueous acidity or alkalinity is required.

The most common definition in wide usage describes an acid as a proton donor and a base as a proton acceptor. Thus water itself serves in both capacities, for the dissociation

$$\underset{\text{acid}}{H_2O} \leftrightarrows H^+ + \underset{\text{base}}{OH^-}$$

$$\underset{\text{base}}{H_2O} + H^+ \leftrightarrows \underset{\text{acid}}{H_3O^+}$$

shows that water supplies a proton and another molecule then takes it up. In this case the reaction contains both acid and base, and both are always found paired in any chemical process involving proton transfer.

Typical acids and bases are illustrated in the following reactions:

Acid	Base	Acid	Base
HCl	$+ H_2O$	$\leftrightarrows H_3O^+$	$+ Cl^-$
CH_3COOH	$+ H_2O$	$\leftrightarrows H_3O^+$	$+ CH_3COO^-$
H_2O	$+ NH_3$	$\leftrightarrows NH_4^+$	$+ OH^-$
H_3O^+	$+ OH^-$	$\leftrightarrows H_2O$	$+ H_2O$
HCl	$+ NH_3$	$\leftrightarrows NH_4^+$	$+ Cl^-$
H_2CO_3	$+ OH^-$	$\leftrightarrows H_2O$	$+ HCO_3^-$
HCO_3^-	$+ OH^-$	$\leftrightarrows H_2O$	$+ CO_3^=$

In each case the acid transfers a proton to the base to form another pair doing the same for the reverse reaction, so that acid plus base yields another acid plus another base. All the illustrative reactions save one involve water. And although there are many non-aqueous acid-base reactions, they are seldom encountered in biology because of the universal distribution of water. Water reacts first with both acid and base before they react with each other. Thus in aqueous solution

$$HCl + H_2O \leftrightarrows H_3O^+ + Cl^-$$
$$H_2O + NH_3 \leftrightarrows NH_4^+ + OH^-$$

and finally

$$H_3O^+ + OH^- \leftrightarrows 2H_2O$$

Acids differ in their tendencies to donate protons. Those with relatively strong tendencies are strong acids, and those with relatively less ability to lose protons are weak. Hydrogen chloride is a strong acid because it transfers essentially all its protons when placed in water.

$$HCl + H_2O \rightarrow H_3O^+ + Cl^-$$

Hydrochloric acid becomes a strong acid because of the high proportion of hydrogen ion present, this ion in turn being a powerful proton donor. In aqueous systems, the strong acids transfer most of the available protons to water, forming a high proportion of hydrogen ions. Weak acids fail to do this, and their aqueous dissociations are written as reversible equilibria.

$$CH_3COOH + H_2O \leftrightarrows H_3O^+ + CH_3COO^-$$

In this typical case only about 1 per cent (depending on the concentration and temperature) of the molecules of acetic acid has reacted with water. Such behavior is characteristic of weak acids, many being far weaker than even acetic acid.

In a similar way there are weak and strong bases. Ordinarily the strong bases always exist in ionic forms even in the absence of water, and only one of the ions present is the actual base. For example, sodium hydroxide in all ordinary states is composed of sodium and hydroxyl ions, the latter actually functioning as the base although the compound as a whole is commonly called a base. In fact, hydroxyl ion is a very powerful acceptor of protons and thus a strong base. Weak bases have a relatively limited affinity for protons, as illustrated by chloride ion, which cannot compete effectively with water for protons in hydrochloric acid solutions. Aqueous ammonia is a weak base because only about 1 per cent of the ammonia molecules is capable of taking protons from water.

$$H_2O + NH_3 \leftrightarrows NH_4^+ + OH^-$$

Some acids can donate two or more protons, and some bases can take up two or more. Phosphoric acid and the corresponding phosphates serve as examples.

$$\begin{array}{lcccccl} \text{Acid} & & \text{Base} & & \text{Acid} & & \text{Base} \\ \hline H_3PO_4 & + & H_2O & \leftrightarrows & H_3O^+ & + & H_2PO_4^- \\ H_2PO_4^- & + & H_2O & \leftrightarrows & H_3O^+ & + & HPO_4^{=} \\ HPO_4^{=} & + & H_2O & \leftrightarrows & H_3O^+ & + & PO_4^{\equiv} \\ \hline \end{array}$$

Thus H_3PO_4 may yield up to three protons, depending on the acceptor present, and $PO_4^{\equiv}$ may acquire up to three protons, depending on the donor present. Each step is a discrete process with a characteristic equilibrium constant analogous to that for water.

$$K_1 = \frac{[H_2PO_4^-][H_3O^+]}{[H_3PO_4]} = 7.5 \times 10^{-3} \qquad \text{(at 25°C.)}$$

$$K_2 = \frac{[HPO_4^=][H_3O^+]}{[H_2PO_4^-]} = 6.2 \times 10^{-8}$$

$$K_3 = \frac{[PO_4^\equiv][H_3O^+]}{[HPO_4^=]} = 4.8 \times 10^{-13}$$

pH

In aqueous systems the dissociation constant of water automatically fixes the concentration of hydrogen ion when the concentration of hydroxyl ion is known. Thus for simplicity only one concentration is usually stated, that of the hydrogen ion. In practice the range of concentrations is so great that the use of a linear scale for plotting is out of the question. The common usage of powers of 10 for expressing concentration leads to the logical scale condensation of logarithms to the base 10. Thus

$$\log_{10} 10^{-4} = -4$$

and to avoid the negative sign in plotting the signs are changed to

$$-\log_{10} 10^{-4} = 4$$

When the concentration involved is that of the hydrogen ion, this operation defines the pH.

$$\text{pH} \equiv -\log\,[H_3O^+]$$

in a solution 10^{-4} molar with respect to H_3O^+, the pH is 4.

Other correlations are shown in Table 2–2 for integral pH values. Thus, unit change in pH alters both hydrogen and hydroxyl-ion concentrations by a factor of 10 and in opposite directions. Use of the pH scale simplifies writing and especially the graphical representation of the effect of the concentrations of these ions on chemical and biological processes.

Buffers

Materials resisting changes in pH are called buffers. They function by accepting added protons (usually from hydrogen ions) or by supplying them when a base is added. In this way changes in pH are minimized. The mechanism of buffer action is the same in all aqueous solutions and depends upon the presence of either a weak acid, a weak base, or both and usually also upon the presence of a salt of the

TABLE 2–2. Relationship between Hydrogen-ion Concentration and pH at 25°C

Hydrogen-ion Concentration, moles/liter		Hydroxyl-ion Concentration, moles/liter	pH	Character
1	$1 = 10^{0}$	10^{-14}	0	Acidic ↑ Increasing acidity (decreasing basicity)
1/10	10^{-1}	10^{-13}	1	
1/100	10^{-2}	10^{-12}	2	
1/1,000	10^{-3}	10^{-11}	3	
1/10,000	10^{-4}	10^{-10}	4	
1/100,000	10^{-5}	10^{-9}	5	
1/1,000,000	10^{-6}	10^{-8}	6	
1/10,000,000	10^{-7}	10^{-7}	7	Neutral
1/100,000,000	10^{-8}	10^{-6}	8	Alkaline ↓ Decreasing acidity (increasing basicity)
1/1,000,000,000	10^{-9}	10^{-5}	9	
1/10,000,000,000	10^{-10}	10^{-4}	10	
1/100,000,000,000	10^{-11}	10^{-3}	11	
1/1,000,000,000,000	10^{-12}	10^{-2}	12	
1/10,000,000,000,000	10^{-13}	10^{-1}	13	
1/100,000,000,000,000	10^{-14}	$1 = 10^{0}$	14	

acid or base. A mixture of sodium acetate and acetic acid serves as an illustration. In solution the following species are present:

$$CH_3COO^- + Na^+$$
$$CH_3COOH + H_2O \leftrightharpoons CH_3COO^- + H_3O^+$$

When Na^+OH^- is added,

$$OH^- + H_3O^+ \leftrightharpoons 2H_2O$$

disturbing the equilibrium dissociation of acetic acid to form more acetate and hydrogen ion in satisfaction of the dissociation constant. In this way hydrogen ions lost by surrender of protons to hydroxyl ions are largely replaced. As a result the pH change is quite slow until most of the acetic acid has been converted to acetate ion. Addition of more hydroxyl ion then changes the pH extensively. This buffer system is effective then in one pH range but not others. The dissociation constant of the acid determines the effective range, so other weak acids would be used in other ranges.

It is readily apparent that acetic acid itself is a good buffer when hydroxyl ion and other bases are being added. However, it is not effective when strongly acidic solutions are added instead, for the large quantity of hydrogen ion quickly combines with the small amount of acetate ion, leaving much hydrogen ion, and the pH changes markedly. When the salt of the acid has been included, however, a large supply of anion is available, and the system is capable of accepting protons in quantity. Thus a mixture of weak acid (or base) and its salt resists change in either direction.

The life processes involve proton transfers. Hence pH could vary widely and fatally since many critical materials break down at extremes of pH. Such changes are prevented by naturally occurring buffer systems of many kinds, but all function by the general mechanism illustrated above.

Gases in Solution

The discussion of the natural transport of gases has already emphasized that gas molecules are dispersed by diffusion in liquids. The resulting solutions then participate in gas exchanges and the like. Quantitatively, rates of diffusion depend upon concentration gradients which are concentration differences in different parts of the system. Such differences in concentration depend partly upon the amount of gas dissolving, which depends in turn upon the pressure of the gas in contact with the solution.

For dilute solutions the concentration of gas dissolved is directly proportional to the pressure of that particular gas exerted on the solution. At higher pressures more gas dissolves, and biological circulatory and diffusion systems can transfer more of it since higher concentration gradients become possible.

The effect of high altitudes on human beings may be cited as an example of the process. People ascending high mountains or flying in planes commonly show symptoms of oxygen deficiency, although there are individual variations in the nature and severity of the manifestations. The basic cause arises from a progressive decrease in air pressure with increasing altitude. And since the composition of air changes but little with altitude, the pressure of oxygen decreases, diminishing the amount dissolving in the blood. Thus the transfer rates decline and the respiring cells run short, particularly in the brain and in the voluntary muscles during exercise. To some extent the human physiology can compensate for the low oxygen pressure, given time for adaptation. Persons with normal hemoglobin synthesis accumulate extra red blood cells, and although each cell transports less oxygen than it did at sea level, the increased cell count helps bring up oxygen transfer. Individuals born and reared at exceptionally high altitudes have an additional tissue adaptation whose nature is not yet understood but which is apparently beyond the physiological capacity of human beings from low altitudes.

In a similar way carbon dioxide deficiencies may be demonstrated for certain microorganisms. In these cases a low pressure of carbon dioxide on the medium leads to too low a concentration in solution, and the cells fail to multiply and may be fatally affected. Such considerations apply to all gases involved in biological exchanges, even those given off, for an exceptionally high pressure of a waste gas can lead to toxic concentrations. Furthermore, sudden large decreases in pressure sometimes allow dissolved gas to form bubbles causing disturbances known as the bends, one of the principal hazards of deep diving.

COLLOIDAL SYSTEMS

When a solution of sodium chloride is placed in a collodion bag and the bag immersed in water, the salt dialyzes rapidly through the collodion film and appears in the water in increasing concentration. However, if starch is suspended in water, none passes through a collodion membrane. Presumably this difference in behavior depends upon differences in size. Generally speaking, ordinary solutions involve solute particles with diameters of less than 1 to 2 millimicrons, 1 to 2 x 10^{-6} mm. Dispersions of particles exceeding about 200 millimicrons are known as coarse dispersions. The intermediate region includes the colloidal systems with particles of intermediate size. Both size limits are quite arbitrary, and the properties of each group merge gradually into those of the neighboring groups.

Among the colloidal solutions there are many similar to the ordinary or true solutions in that the dispersed phase is a homogeneous molecular dispersion. Suspensions of soluble proteins and large carbohydrates fall into this category. On the other hand, dispersed aggregates of soaps, clays, and the like are also colloids. Hence the classification is merely one of size and does not correspond closely to groupings of chemical or other physical properties. However, colloids are particularly important in certain respects discussed below.

Formation

Those colloids whose individual molecules place them in this size range are obviously the result of natural and biological syntheses. Many of these processes have not yet yielded to the chemist, but others have been duplicated in the laboratory, for example, the production of rubber, plastics, and polymers. In these materials the principal forces holding the giant molecules together are ordinary valence forces.

The other colloids consist of aggregates of smaller molecules, not necessarily all alike, held together by attractive forces often called the secondary forces. These interactions are strong enough to yield rather large particles, although there occasionally is equilibrium between particles of different sizes. Sometimes the colloidal system is unstable and the individual particles coalesce, forming a coarse dispersion and perhaps precipitating altogether. In other cases they may separate into the component molecules, yielding an ordinary solution.

Clearly either of the foregoing changes could be reversed to form dispersions of the colloidal type. Thus grinding with machines called colloid mills can break large particles into those of colloidal size. Moreover, certain added materials induce a spontaneous breakdown called peptization with formation of a colloidal system. Conversely, a reduction in temperature, prolonged standing in solution, a suitable chemical reaction or agent, or the addition of another solvent may cause molecules to associate. Thus there are two general types of methods, those causing dispersion and those causing aggregation, and there are many variations for either method.

Stabilization and Precipitation

These two types of treatment of colloidal systems are complementary. When stability is desired, all factors provoking precipitation are avoided, and conversely. In general, stabilization is aided by (1) an optimum choice of temperature, (2) addition of stabilizing or peptizing agents, (3) keeping the system dilute, and (4) using a good solvent. In the first instance, many colloidal solutions are destabilized by freezing and thawing or by heating, especially boiling. In the second, peptizing agents prevent further aggregation by coating the surfaces of the particles and making them repel each other or at least preventing adhesion when they do collide. In the third, in a dilute system collisions are less frequent, and when an equilibrium exists, dilution favors the smaller aggregates below the range of precipitation. Finally, solvents corresponding somewhat in structure and polarity to the dispersed phase help reduce the forces of aggregation.

Precipitation is promoted by reversing the above procedures, as by freezing or boiling, omitting peptizers, concentrating, and adding poor solvents. In addition, precipitation results when suitable chemical agents are added, heavy metals, acids or bases in some cases, or other substances changing the particle surface to allow adhesion on contact.

Adsorption

Many systems show a relatively high concentration of the solutes present at the various surfaces between phases. This selection phenomenon is called adsorption and in its general form may be illustrated by the increased concentration of certain surface-active materials in the film at the solution-air interface. Frequently adsorption, sometimes called sorption, involves neither particles nor surfaces of colloidal dimensions, although the adsorbed layers are usually thin, from one to a few molecules in thickness. However, adsorption is commonly discussed in connection with colloidal systems, partly because colloid chemists did much of the early work on adsorption and partly because the colloidal systems present relatively great surface areas on which adsorption can occur.

With a given weight of material total area increases with increasing subdivision. Thus a colloid presents a vastly greater area for concentration of material than does an equal weight of a coarse dispersion. Naturally dispersion of the adsorbing material into the individual molecules increases the total area still more. However, it seems that small molecules often possess secondary force fields somewhat different from those of aggregates of these same molecules, and adsorption diminishes if the particles get too small.

Classical valence bonds do not seem to participate in adsorption. Instead secondary forces like those between the molecules in a liquid or the interactions between the dipolar molecules of water lead to adsorption. An accurate general theory of adsorption is still not available, but a number of empirical expressions have been proposed. Usually called adsorption isotherms, the equations can be applied to certain aspects of adsorption in certain cases only.

The extent of adsorption is known to depend upon the natures of the solvent, adsorption surface, and the material adsorbed. It usually decreases with increasing temperature and may be affected by violent stirring. Additional solutes and changes in pH, dielectric constant, and concentration modify adsorption, often drastically.

The phenomenon is used to concentrate all manner of materials, gases, liquids, or dissolved solids. These concentrations are used in isolation, purification, separation, deodorizing, detoxifying, decolorizing, and many other laboratory and practical processes. Adsorption may be so effective as to concentrate the minute traces of nutritional materials in distilled water enough to permit the growth of microorganisms at the surface of the suspended adsorbing material.

Colloids in Biology

Many giant molecules known as macromolecules function biologically. They include blood proteins, cellular enzymes, starch, glycogen, viruses, nucleic acids. All are necessary and all fall in the colloidal size range. In addition some of the fats may be transported in body fluids as aggregates of or near colloidal size. Cells contain many suspended particulates, some of colloidal dimensions, that are believed to be aggregates active in cellular reactions. In addition, blood colloids, for example, adsorb small solutes, and the complexes circulate, serving as reservoirs of the adsorbed materials.

Also there are non-crystalline solid-like materials having some colloidal properties. Gels like gelatin and jelly possess some sort of interlocking structure that includes a great deal of water and are really neither liquid nor solid. The protoplasm of living cells is viscous like the gels and resembles them. So does muscle tissue. Some hard materials like horn, finger nail, and glue appear to be concentrated gels of high tensile strength. A number of these biological colloids will be considered in greater detail later.

COARSE DISPERSIONS

Interfacial Tension

The coarse dispersions include all systems of two or more phases with the particles exceeding about 200 millimicrons in diameter. At each surface separating the various phases of the system there is a force resembling the surface tension of the boundary surface between a liquid and air. In fact the ordinary surface tension of liquids is but a special case of this general type of force called interfacial tension.

There are five different classes of phase boundaries, gas-liquid, liquid-liquid, gas-solid, liquid-solid, and solid-solid. All represent zones of special force with the consequent possibilities for interactions with solutes and resulting adsorption. The first case has already been discussed. Of the others, the last three are thought to exist but no methods of measurement have ever been devised. Presumably the forces involved lead to adsorption, but little more can be said.

However, liquid-liquid interfaces definitely possess tensions that can be measured by using modifications of some of the techniques developed for gas-liquid interfaces. The measured interfacial tensions have correlated with adsorption and play major roles in studies of emulsions. These coarse dispersions are suspensions of one liquid in another and may be indefinitely stable, or they may be stabilized by emulsifying agents. The latter are materials adsorbed at the interface; by their presence they prevent cohesion of the dispersed droplets.

Common emulsions include milk, in which liquid fat is dispersed in an aqueous phase; butter, in which aqueous phase is dispersed in fat; and the microscopically visible oil droplets seen in blood after butter has been eaten, as well as ointments, salad dressing, and cosmetic creams.

Populations and Ecology

Little is known about the special physical and chemical properties of coarse dispersions, although many branches of engineering are largely concerned with them. The well-developed techniques for studying ordinary solutions apply to some extent to colloidal systems but almost not at all to coarse dispersions. Hence few natural laws are known describing the behavior of these systems, and theoretical development has hardly begun.

Yet in one special area a start has been made in work on coarse dispersions. A group of individuals of a given species is called a population, or if this group exerts a degree of conscious self-control it may be called a society. In the latter case the social sciences study the communal behavior of the group, the effect of the society on the individual and the converse, the result of environmental or psychological stresses, economics, and so forth. The more general problems of populations consider some of these same aspects. In one respect the general problems of populations are perhaps a little simpler than those of societies, since populations may show little conscious group self-control. Individuals may function consciously on occasion but with little real regard for the entire group. Thus chance conditions produce more nearly reproducible results. Population studies might involve human beings, rats, protozoa, or almost any other form of life, including even non-motile types like plants.

One special phase of this field concerns the interactions of populations. When the environments of two populations overlap, one group affects the other directly or indirectly. The complete history of a group of plants is directly affected by the presence of grazing animals. Even those not eaten may be trampled. One or more species able to survive repeated damage will be automatically favored, filling in vacancies left by species unable to withstand the stress. The reverse is also true, for the nature of the plants will determine the presence or absence of grazing animals. In the absence of suitable vegetation the vegetarians cannot invade the environment, and in turn the carnivores are excluded.

Since all environments contain more than one population, there are always interactions. The study of these interactions is called ecology and is now pursued partly as a scientific problem and partly in the hope of financial returns from improvements in crop yields, soil conservation, livestock industries, fishing for both pleasure and profit, and lumbering. Man is, of course, one of the earth's greatest ecological factors, owing to his size, broad range of activity, and great numerical abundance.

As examples of ecological phenomena consider the epidemics of disease resulting when population density rises high enough for contagion to spread by contact. Note also the multiplication of particular insects attacking certain crops cultivated intensively. A still more far-reaching example involves the rabbits of northern Canada. In this region rabbits multiply rapidly, the population following cycles of about seven years, whereupon the numbers are great enough to support an epidemic which sweeps off a high proportion of the individuals. The remainder reproduce rapidly, starting another cycle. However, shortly after the expansion of the rabbit population begins, the lynx population also starts to increase since the food supply is plentiful. But when the majority of the rabbits die of disease, introducing a great competition among lynx for the remainder, the situation becomes so acute that lynx die, migrate to other areas, and simply fail to produce young. Hence the lynx population also declines a year or two after the epidemic that decimates the rabbits. Other animals depending directly or indirectly on the rabbits show similar cycles, shifted from that of the rabbits according to the time required for pressure to develop in each population. Even man is affected, since trappers find regions profitable or not depending on the cycles of the fur-bearing animals and go elsewhere when fox, lynx, wolverine, marten, and all the others are few in number.

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Carbohydrates

3

Living matter is made up of several large classes of compounds. One of these groups includes the substances known as carbohydrates. The compounds thus designated in actual practice are so varied that a suitable definition really does not exist. Perhaps the simplest statement says that carbohydrates are polyhydroxy aldehydes or ketones or are condensation products involving the carbonyl groups of such aldehydes or ketones. These condensation products are formed by loss of water and give rise to giant molecules in certain cases. Occasionally other groups are present as in the amino sugars. Actually the term *carbohydrate* is applied to substances of diverse chemical, physical, and biological properties. But ordinarily all may be hydrolyzed to molecules of relatively similar chemical behavior, the polyhydric aldehydes or ketones.

OPTICAL ISOMERISM

Optical Rotation

As an electromagnetic radiation, light vibrates in directions perpendicular to the direction of propagation. When light strikes a flat surface at an angle, the light vibrating in one plane is preferentially reflected and is said to be plane polarized. Light transmitted through most crystals is sorted into two components, each plane polarized. By special cutting and cementing of the crystal, one component can be absorbed, leaving a beam of light with a single plane of polarization. A few materials do this automatically, such as the mineral tourmaline and the industrial product Polaroid.

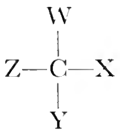
Whatever the source of the polarized light, its properties are the same. Among these properties is rotation of the plane of polarization when the light passes through any one of a variety of crystals or through a solution of any one of a variety of substances. This rotation is called optical rotation and is of interest in biology and related fields because solutions of many naturally occurring organic compounds, including carbohydrates, rotate the plane of polarization. An instrument called the polarimeter detects and measures the magnitude of the effect. It contains a polarizer to provide the plane-polarized light, a solution chamber, an analyzer crystal to compare the position of the plane of polarization with and without the solution, and optics and scales for observing and measuring the rotation.

Optical rotation depends upon the nature of the dissolved substance, the solvent, pH, other solutes, temperature, concentration, and wave length of the light. Although the effects of these variables have all been studied, a general theoretical consideration is not possible. However, if other factors are kept constant, the optical rotation is a characteristic of the substance and may occur in either a clockwise or a counterclockwise direction. More important, the rotation provides certain correlations with molecular structure.

Optical Activity and Molecular Configuration

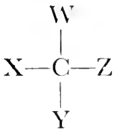
Optical rotation by crystals is often correlated with the relative arrangements of molecules or ions in crystals rather than specifically with the structures of the molecules themselves, although in many solids both factors may be involved. However, in solution only the molecular effects remain, and solutions thus assume primary importance in work on molecular structure. Hence solutions or liquids rotating the plane of polarized light contain molecules said to be optically active.

Optically active molecules contain at least one characteristic configurational arrangement. The type of most interest here is described as a carbon atom with four different atoms or groups attached. In such a case two different fixed arrangements are possible with these same groups. Represented in two dimensions with C as the carbon atom and W, X, Y, and Z as the different atoms or groups, the two arrangements are



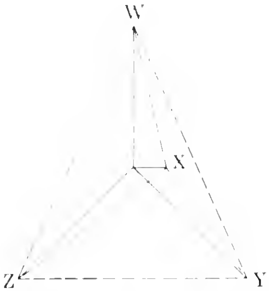
$$\begin{array}{c} \mathrm{W} \\ | \\ \mathrm{Z—C—X} \\ | \\ \mathrm{Y} \end{array}$$

and

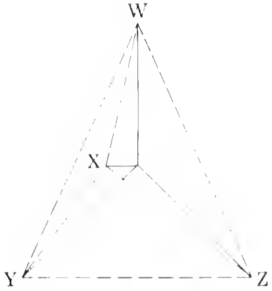


$$\begin{array}{c} \mathrm{W} \\ | \\ \mathrm{X—C—Z} \\ | \\ \mathrm{Y} \end{array}$$

These structures appear to be mirror images of each other. Rotation of one cannot yield the other, and they represent different compounds. Actually, of course, the four bonds drawn above do not lie in a single plane but extend at angles giving the relatively regular pyramidal structure:



and

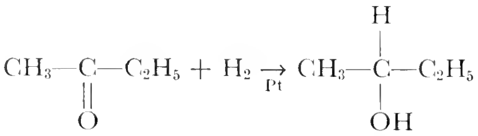


Shown in perspective, the chemical bonds are heavy lines connecting the groups with the carbon atoms located at the points of intersection of these lines. The dashed lines are included to show the overall pyramidal shape. In the particular orientation chosen, the pyramids are lying on one edge in the plane of the paper with faces partly opened toward each other. In each case the bond to the group X projects at an angle through the plane of the paper and away from the observer. Neither structure can be superimposed on the other merely by reorientation. For example, if the structure on the left is flipped over to place groups W, Y, and Z on those of the right-hand structure, group (or atom) X projects at an angle out of the paper toward the observer. Thus compounds differing in this way are said to have different configurations about the central carbon atom and are called optical isomers when the atoms or groups differ between the structures only in their spatial arrangements.

Polarized light transmitted by a solution of one of these forms is rotated one way and in the other direction by a solution of the other optical isomer. The magnitudes of rotations are the same since the groups themselves are identical. The difference in direction is designated with a plus sign, the letter *d*, or the prefix *dextro* for clockwise rotation, and a minus sign, the letter *l*, or the prefix *levo* for counterclockwise rotation. Since the substituent groups of the two isomers are identical, the chemical properties of the compounds are also identical in many cases. Likewise, the physical properties other than optical rotation are often identical. However, the biological properties are usually different, often spectacularly so. Hence, this particular type of structural consideration is important in the study of biochemistry.

When carbon atoms are attached to two or more like groups or atoms, only one configuration is possible, and the carbon atoms are described as symmetrical. In contrast the carbon atom with four different substituents is asymmetric, and examination of the structural formula of a compound for such asymmetry reveals the possibility of optical isomerism.

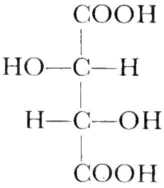
Many simple symmetrical compounds undergo standard types of laboratory reactions with the formation of a center of asymmetry, as, for example:



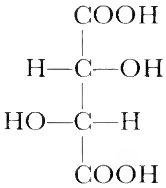
$$CH_3-\underset{\underset{O}{\|}}{C}-C_2H_5 + H_2 \xrightarrow[Pt]{} CH_3-\overset{\overset{H}{|}}{\underset{\underset{OH}{|}}{C}}-C_2H_5$$

Carbon two no longer possesses symmetry, yet the product has no detectable optical activity. In reactions of this type the chance of forming one isomer equals the chance for the other, and equal quantities of both are formed. By this means a mixture is formed with one half rotating polarized light one way and half the other, a net effect of zero rotation. Thus optical isomerism does actually result in such cases as the above, but optical rotation does not. Such a system is called a racemic mixture and is readily duplicated by combining equal quantities of the two pure isomers. It is possible by using specially developed methods to separate (resolve) a racemic mixture into the component isomers, although the standard practices employed in the separation of otherwise different compounds ordinarily do not work.

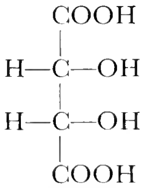
In those substances possessing more than one asymmetric carbon atom, certain special situations arise. If two asymmetric carbons in the compound have like substituents, one half of the molecule may be the reverse of the other half. Tartaric acid is the standard example.



(−)-tartaric acid



(+)-tartaric acid

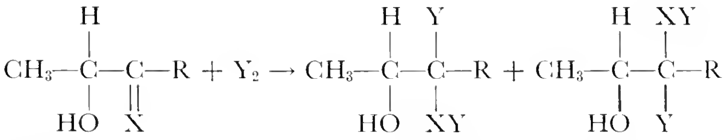


meso-tartaric acid

In the third isomer, one group is believed to rotate the light one way and the other to reverse the rotation exactly, so that this isomer as a whole is optically inactive by internal compensation. Such substances are called meso-isomers. They occur as symmetrical molecules having more than one asymmetric carbon atom. In other words, the molecule as a whole is symmetrical whereas certain of the individual carbon atoms are not.

Most compounds with more than one asymmetric carbon have different groups on the halves of the molecule and cannot exhibit the symmetry of meso-isomers. They may, of course, occur as racemic mixtures corresponding to an equal mixture of (+)- and (−)-tartaric acids. Moreover, the synthesis of such compounds normally leads to these optically inactive mixtures.

However, when a compound possessing an asymmetric center undergoes reaction to form a second center of asymmetry close by, there is a directive influence exerted by the first center. In other words, an asymmetric carbon atom in a molecule tends to control the configuration of the second center of asymmetry introduced later. Naturally, the use of a racemic mixture in the reaction will yield a racemic product because both the original isomers possess equal and opposite directive effects. However, when a single isomer is placed in the reaction, a preponderance of one form results in these asymmetric syntheses. The general result may be illustrated by the reaction shown, where one product is more abundant than the other.



$$CH_3-\underset{HO}{\overset{H}{C}}-\underset{X}{\overset{}{C}}-R + Y_2 \rightarrow CH_3-\underset{HO}{\overset{H}{C}}-\underset{XY}{\overset{Y}{C}}-R + CH_3-\underset{HO}{\overset{H}{C}}-\underset{Y}{\overset{XY}{C}}-R$$

As might be expected, the relative quantities of the isomers depend upon the original asymmetric structure, the nature of the reaction, and the proximity of the second center of asymmetry to the first. When the two are far apart there is little or no effect. On the other hand, the presence of several centers of asymmetry is believed to enhance the control of configuration. Moreover, the intervention of an optically active catalyst can control the configuration about centers of asymmetry. Both of these last effects in combination are believed to bring about the high degree of optical specificity found in biological materials.

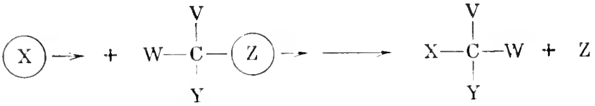
In the foregoing illustrative case, the formation of mirror images is impossible, a fact believed to account at least in part for such asymmetric synthesis. This situation arises from the fact that a single isomer was used in the reaction and the configuration about this group is maintained throughout the reaction. The process may be schematically represented, using only the signs of rotation and starting with the positive isomer

$$+ \rightarrow + - \text{ and } ++$$

showing that the products cannot be mirror images of each other.

Isomers of this type are called diastereoisomers and differ in their physical, chemical, and especially biological properties. They may differ considerably in their melting points, solubilities, and the like. They may differ somewhat in their chemical reactivities or at least in reaction rates because the differences in the relative locations of groups slightly alter the arrangements of electrons about the molecules as a whole. Biologically, diastereoisomers are usually quite different. One may be a digestible foodstuff and the other not, owing in general to the interaction or lack of it with optically active catalysts. Such interactions require a particular optical configuration; others do not fit.

Under certain conditions a bond between an asymmetric carbon atom and a substituent is broken and re-formed. Often there is a sufficient lapse of time between rupture and repair for the remaining groups to alter their relative positions. Then, when the bond in question is re-formed, either the original configuration may result or the opposite one. This process is called racemization and leads to a racemic mixture. Racemization is quite common when reactions break one or more of the bonds of the asymmetric carbon atom. Sometimes a different phenomenon is observed. When a bond is broken, and a new atom or group approaches for attachment to the carbon, the electronic nature of the approaching structure requires that it enter only from the direction opposite that of the loss of the group removed. This requirement forces a rearrangement of the remaining substituents and leads to a reversal of configuration called a Walden inversion. The reaction may be shown schematically in two dimensions as follows:



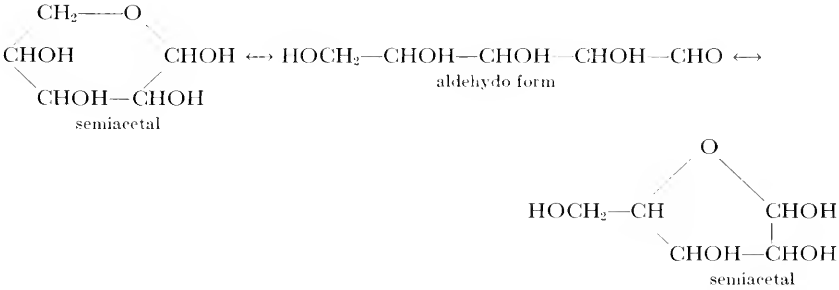
$$\textcircled{X} \rightarrow + \; W{-}\underset{Y}{\overset{V}{C}}{-}\textcircled{Z} \rightarrow \;\longrightarrow\; X{-}\underset{Y}{\overset{V}{C}}{-}W + Z$$

This type of rearrangement has long been studied in laboratory reactions and is now known to occur in biochemical processes, particularly in the interconversion of carbohydrates. Probably Walden inversions may turn out to be more widespread in living systems than is now suspected.

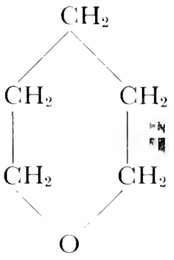
GENERAL PROPERTIES OF CARBOHYDRATES

Structures

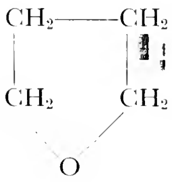
Carbohydrates are characterized by the presence of carbonyl groups, often in acetal or semiacetal form, and hydroxyl groups. The carbonyl groups are of the aldehyde or ketone type and typically react with alcoholic hydroxyl groups to form semiacetals, also called hemiacetals. Since carbohydrates usually possess suitably located hydroxyl groups on the same molecule as the carbonyl, the addition reactions are internal to form cyclic derivatives. Natural carbohydrates are largely found in these cyclic forms and might be represented thus:



Considerations of ring strain lead one to expect both of the systems above, and semiacetals of both types can be made. Six-membered rings of this type are derivatives of tetrahydropyran; five-membered types, of tetrahydrofuran. Carbohydrates are known correspondingly as pyranose or furanose forms. Many natural examples possess the first ring system, a few the other, and some complex cases both, although pyranose forms predominate.



tetrahydropyran



tetrahydrofuran

Semiacetals readily undergo reactions with alcohols to form acetals and water. When HOR is any alcohol including another carbohydrate, the product is called a glycoside, but when ROH is limited to the carbohydrates, a larger carbohydrate is obtained. Repetition of the latter process yields carbohydrates of great molecular size. Obviously

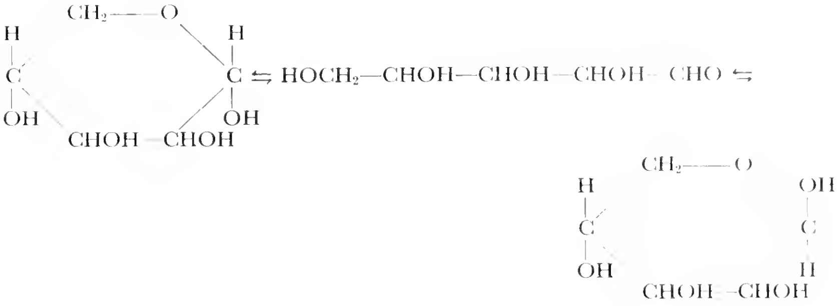
the reaction to form carbohydrates is difficult to control in the laboratory because both reactants are semiacetals and both possess several hydroxyl groups, so a diversity of similar reactions is possible, all leading to acetals.

Mutarotation

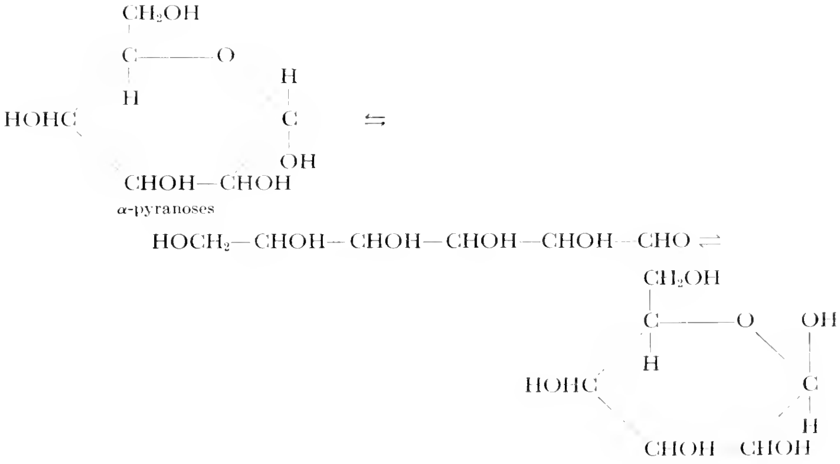
The formation of the ring systems of the semiacetal forms of carbohydrates introduces a center of asymmetry at the carbon atom originally bearing the carbonyl group. Thus two isomers become possible for each carbonyl structure. These isomers possess somewhat different properties and are usually known as the α and β forms. The conventional designation depends on the configuration of the semiacetal group relative to the configuration of the asymmetric carbon most remote from this semiacetal group.

When writing the structures of carbohydrates in the forms used here, at least two different conventions must be employed. If the asymmetric carbon atom of highest number (most remote) carries a free hydroxyl group, the α isomer is written with this hydroxyl group on the same side of the ring as the hydroxyl group of the semiacetal structure. The β isomer is shown with these two hydroxyl groups on opposite sides of the ring; see below. If the hydroxyl group of the asymmetric carbon atom of highest number is involved in the semiacetal linkage, the attached hydroxymethyl group and the hydroxyl group of the semiacetal structure are written on opposite sides of the ring for α isomers and on the same side for β isomers. In certain cases not discussed in this book, still other conventions must be employed. Both α and β forms are stable in the solid state and may be synthesized or isolated.

In aqueous solution, pyranose and furanose rings open reversibly. A solution of a pure α-pyranose, for example, changes steadily in optical rotation to an equilibrium value representating a mixture of both α and β forms with perhaps a small fraction of the molecules in the carbonyl form, as in the above reactions. The relative preponderance



or


α-pyranoses

β-pyranoses

of the forms depends on the other groups present, these exerting a directive effect favoring one over the other because the other groups are themselves asymmetric. In other words, the α and β isomers are not mirror images but are typical examples of the diastereoisomers discussed on page 54 and as such differ in many of their properties. This equilibrium transition leading to the mixture of isomers is called mutarotation. This term reflects the discovery of the phenomenon by way of changes in the optical rotations of certain carbohydrates.

Mutarotation is quite characteristic of semiacetal but not of acetal linkages. In aqueous solutions of the latter, the equilibrium, if there is one, is so far toward the acetal form that changes are not observed. Thus acetals of the carbohydrate type show no spontaneous tendency to revert to semiacetals or to the acyclic forms. Nor, when acetals are deliberately hydrolyzed to the semiacetals, is reversion to the acetals spontaneous.

Identification of Carbohydrates

The large carbohydrates are either insoluble in water or form colloidal systems and usually cannot be individually identified by the application of general group tests. However, hydrolysis yields the water-soluble component carbohydrate units which are readily detected by means of tests for carbonyl and hydroxyl groups.

Carbonyl tests of value depend upon reduction of heavy-metal ions, reactions with amines or phenylhydrazine, and conversion to furfural derivatives and color tests for these compounds. Hydroxyl groups are seldom directly considered and are usually inferred from water solubility, certain phenylhydrazine reactions, or furfural formation.

Reduction of the complex of cupric ion in alkaline tartrate to insoluble cuprous oxide is the basis of Fehling's test. Various gravimetric and volumetric analyses determine the amount of cuprous oxide produced, correlated in turn with the reducing material present. Carbohydrates with free carbonyl groups or in semiacetal form give positive tests, but carbohydrates with only acetal groups do not. The same response is found with cupric ion in alkaline citrate and cupric ion in acetic acid in Benedict's and Barfoed's tests, respectively. Silver ion is reduced to metallic silver in alkaline solutions containing sugars of the aldehyde type. All the tests of this group depend upon reduction by the carbohydrates. As a result those compounds reacting are known as reducing sugars, the acetal types as non-reducing sugars.

Many aldehydo and keto carbohydrates in either free carbonyl or semiacetal forms react characteristically with phenylhydrazine to form crystalline insoluble derivatives called osazones. In addition, certain aromatic amines form colored compounds with most of the smaller carbohydrates, giving useful spot tests for small amounts. Furthermore, many carbohydrates are readily dehydrated in the presence of mineral acids to form furfural or certain of its derivatives. This new aldehyde reacts in turn with a variety of phenols to give typical colors. The Molisch test employs sulfuric acid for the dehydration and α-naphthol as the phenol. The acid also hydrolyzes acetal groups present and makes this test perhaps the most general of all for carbohydrates.

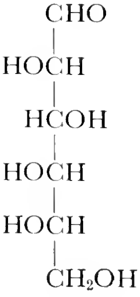
CLASSIFICATION OF CARBOHYDRATES

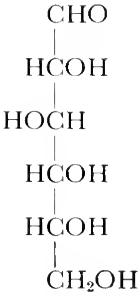
Several different systems are used in classifying carbohydrates, depending on the properties chosen for consideration. In practice, any one of these systems may be used or two or more may be combined to describe as many properties as possible. Some of the information incorporated into these taxonomic schemes has already been mentioned.

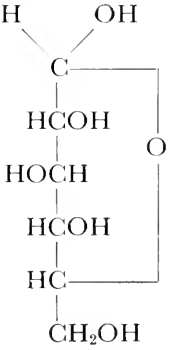
Carbohydrates may be classified according to the nature of the rings of the acetal and semiacetal forms. They may be grouped according to the configuration about the semiacetal (or acetal) atom, the number one carbon. They may be divided into reducing and non-reducing carbohydrates, depending on the presence or absence of free carbonyl or semiacetal groups. The small, water-soluble representatives are also known as sugars, most of them yielding solutions with a sweet taste.

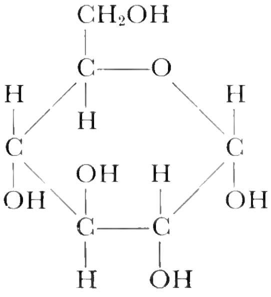
Beyond these descriptive terms are others. One of these is widespread and specifies the number of individual carbohydrate units into which the parent molecule may be hydrolyzed. The root word *saccharide* is used as the suffix and signifies carbohydrate. If the original compound is a simple carbonyl or semiacetal sugar incapable of hydrolysis, it is termed a monosaccharide. When a sugar hydrolyzes into two such monosaccharide molecules, it is a disaccharide, into three a trisaccharide, and so forth. The giant carbohydrate molecules hydrolyze into many monosaccharide units and are called polysaccharides. The classes undergoing hydrolysis may yield only a single kind of monosaccharide, or they may yield more than one kind, depending on the nature of the parent carbohydrate.

With several asymmetric centers present, a variety of diastereoisomers can exist. These forms differ from one another chemically, physically, and biologically and are distinguished by means of different names like glucose and galactose. However, mirror-image sugars are known and are described by optical rotations or more commonly by conventional representations of their configurations. This system is applied to the individual monosaccharides even when they are component parts of larger molecules, never to the larger units themselves. The atom conventionally chosen for the representation key is the asymmetric carbon atom of highest number, always counting from the carbonyl, semiacetal, or acetal group, or the end nearest one of these, along the carbon chain. Using glucose as an example,


L-glucose


D-glucose


α-D-glucose


α-D-glucose

Written in the chain form with the aldehyde group at the top, the highest-numbered asymmetric carbon atom is in the D configuration when the hydroxyl group is written to the right of the chain. Thus the monosaccharide is a D sugar regardless of the configurations about other asymmetric carbons and regardless of the actual optical rotation. In the case of D-glucose both the α and β forms have positive optical rotations in ordinary aqueous solutions, but this is not true for other sugars, D-fructose, for instance. The actual optical rotation depends upon all the structural groups present, the solvent, the temperature, etc., and is of little direct value in classifications on structural grounds.

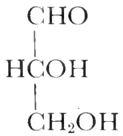
In the pyranose form written like the chain, the oxygen bridge involves carbon atom five. The bridge connects to the atom from the right in the D isomer. Written in this way, the bonds of the oxygen in the ring have no correlation with reality except the points of connection. Hence the right-hand formula on page 59 is preferred as a better representation of the actual relative positions of the groups. Even this portrayal has limitations due to the difficulties of projection from three to two dimensions. Sets of atomic models are very useful in visualizing carbohydrate structures and actually aid in the solution of structural problems.

In addition to the foregoing classifications, monosaccharides are subdivided into ketoses and aldoses when ketone or aldehyde groups, respectively, are present in either free or semiacetal form. Moreover, the monosaccharides are subdivided according to size, depending on the number of oxygen or of carbon atoms. Some systems use one element, some the other, and none are free of difficulties. When oxygen atoms are counted, perhaps the commonest method, a diose might be considered the smallest carbohydrate. The compound is optically inactive,

$$\mathrm{CH_2OH{-}CHO}$$

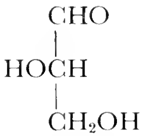
glycolicaldehyde

having no center of asymmetry. The next higher members are the three oxygen compounds shown and are trioses. The ketose has no



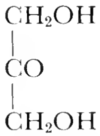
$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HCOH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

D-glycerose



$$\begin{array}{c} \mathrm{CHO} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

L-glycerose



$$\begin{array}{c} \mathrm{CH_2OH} \\ | \\ \mathrm{CO} \\ | \\ \mathrm{CH_2OH} \end{array}$$

dihydroxyacetone

center of asymmetry, and internal semiacetal formation does not occur because of the strain in a three-membered ring, so this first ketose is optically inactive.

These last two systems of classification are often combined to specify both the number of oxygen atoms and the type of carbonyl group involved. Thus the above aldoses are aldotrioses and have in this case the common name glyceraldehyde. Dihydroxyacetone by this nomenclature is a ketotriose, the only such sugar possible in this instance. By analogy, glucose is an aldohexose, and so on for the other monosaccharides.

A few sugars are methyl derivatives; these are simply called methylaldopentose and the like. In one important natural five-carbon sugar, deoxyribose, the second carbon is missing the oxygen atom. This exceptional compound is usually included among the pentoses, nevertheless. Sugars with amino groups are considered as a separate class at present regardless of their relationships with the other carbohydrates.

To summarize, the most common classification in use groups the carbohydrates into mono-, di-, tri-, and polysaccharides. These classes are then divided further, the monosaccharides according to both the number of oxygen atoms and the type of carbonyl group, the disaccharides into reducing and non-reducing sugars, and the polysaccharides according to the sizes of the monosaccharide subunits. For this last classification step the polysaccharides made up of pentoses are pentosans, those made up of hexoses are hexosans.

IMPORTANT CARBOHYDRATES

The number of theoretically possible carbohydrates is astronomical. Fortunately for the chemist and biochemist the number occurring naturally seems to be limited, and these are of the greatest interest, although some others have been synthesized. Even of the known natural carbohydrates, space dictates that only a few can be discussed individually. The choice is quite arbitrary, so the literature must be consulted for anything like a comprehensive treatment of the subject. Moreover, a number of derivatives of carbohydrates play important biochemical roles. Some of these are discussed in later chapters.

L-Arabinose

This important aldopentose is obtained on hydrolysis of a variety of vegetable gums and occurs in legumes in small amounts and in urine in certain pathological states. As is true of most other sugars, it is used by a number of species as both an energy and a carbon source. The optical isomer D-arabinose also occurs naturally in the form of a derivative in aloes and with L-arabinose in the pathological states mentioned above. See the structural formula below.

D-Ribose

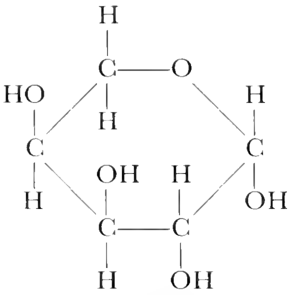
This aldopentose occurs in various derivatives of metabolic interest. D-Ribose is one of the components obtained on hydrolysis of the ribonucleic acids. These macromolecules contain phosphate and organic bases in addition to the sugar. They are essential to life as we know it, although their function is not well established. Certain viruses contain ribose, once again in the form of ribonucleic acid.

D-Deoxyribose

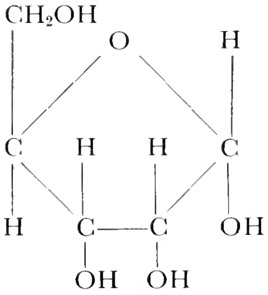
This sugar resembles ribose itself in occurrence. The deoxyribonucleic acids occur in some viruses and in all living cells, where they are involved in cell division and carry the hereditary traits. The nucleic acids are discussed more fully starting with page 120. As indicated by the prefix, this sugar has no oxgyen atom on carbon two but is otherwise like D-ribose.

D-Xylose

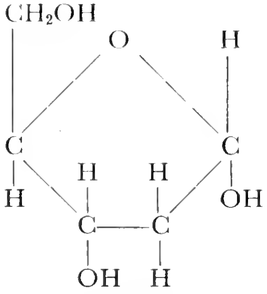
This carbohydrate occurs in wood, straw, and other fibrous materials in combined forms from which it is obtained on hydrolysis. Small



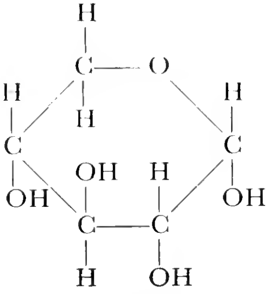
β-L-arabinose



α-D-ribose



α-D-deoxyribose



α-D-xylose

amounts have also been isolated from liver and pancreas. Ruminants probably utilize a portion of the combined xylose of the plants they consume after the polysaccharides containing it are hydrolyzed by the bacteria in the rumen.

D-Glucose

This sugar (also known as dextrose, starch sugar, or corn sugar) is the most abundant of the hexoses. It is found in free form in ripe fruits, sweet corn, and honey, where it is associated with fructose. Glucose occurs in many of the large carbohydrates, including the most abundant examples. It is sweet in taste and is readily metabolized by microorganisms of many kinds and by all higher plants and animals. In the latter, glucose is the usual form of transport for carbohydrates, many others being converted to it before circulation in the blood. Cells put the atoms of glucose to a great variety of uses in the course of their normal biochemical operations. Some of these functions will be outlined later.

D-Fructose

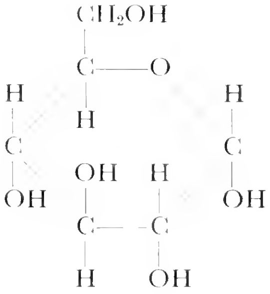
This sugar is a ketohexose and the only really abundant ketose in nature. It is often called levulose or fruit sugar and is widely distributed with D-glucose. It occurs in several of the larger carbohydrates. In the free form fructose is found with the pyranose ring, but in its derivatives the furanose system is common.

D-Galactose

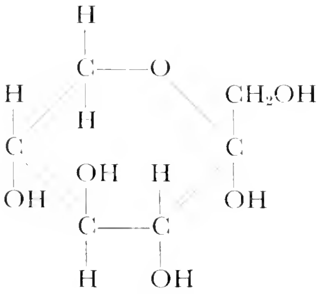
This aldohexose is found largely in combined forms which yield it on hydrolysis. The free sugar is neither so sweet nor so water soluble as glucose or fructose though it is a diastereoisomer of the former. D-Galactose occurs in combination with D-glucose in the disaccharide lactose.

D-Mannose

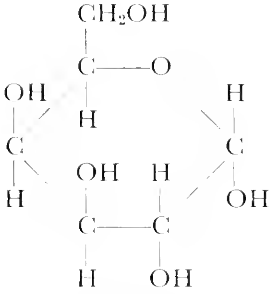
This sugar is another diastereoisomer of D-glucose. It is found principally as a component of mannans, a group of hexosans occurring in fruits like the berries of the mountain ash tree. This sugar is also abundant in vegetable ivory, the endosperm of the nut of a low-growing palm of tropical America. The ivory is used in making ornaments and buttons. The name *mannose* is derived from manna, a term applied to any one of the fruits or plants rich in this carbohydrate.



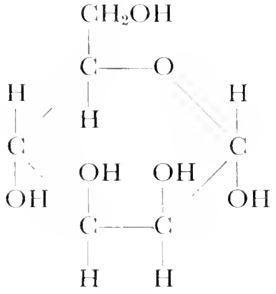
α-D-glucose



α-D-fructose



α-D-galactose

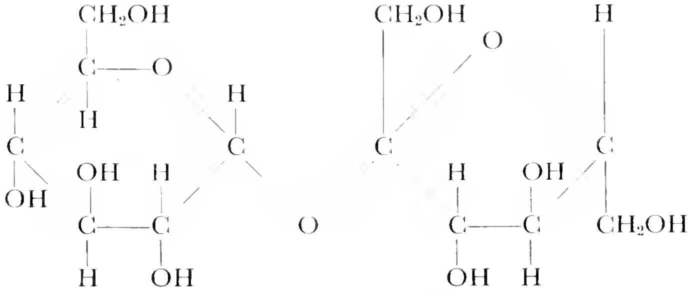


α-D-mannose

Sucrose

This disaccharide is also known as cane sugar, beet sugar, or saccharose. It is the most widely distributed in free form of all the sugars of this group and occurs in most green plants. The best commercial sources are the sugar cane, beet, palm, and maple and sorghum cane.

Nectar of flowers is rich in sucrose and is the raw material for honey. Bees concentrate the solution and hydrolyze the sucrose to a mixture of glucose and fructose. Thus these two monosaccharides are combined in sucrose with elimination of a molecule of water. Moreover, the tests for reducing carbohydrates are negative with sucrose, indicating that both potential carbonyl groups are involved in the acetal linkage. Chemical studies on sucrose show that the fructose portion is



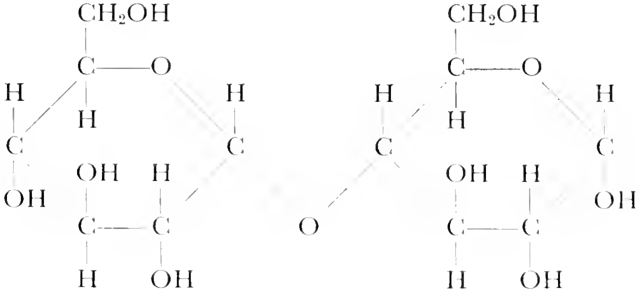
1-α-D-glucosido-2-β-D-fructose

in the furanose form and the glucose portion in the pyranose form. The structural formula is shown on page 61 where the numbers designate the positions linked via the oxygen connecting the two monosaccharide fragments.

Sucrose cannot show mutarotation since there is no semiacetal structure. Hence sucrose exists in but one form and is readily crystallized. An aqueous solution at 20° C. rotates the plane of polarization clockwise. When the sucrose has been hydrolyzed, the direction of optical rotation is reversed. This change arises when the strong counterclockwise (levo) rotation for D-fructose is added algebraically to the relatively smaller dextro rotation of an equivalent solution of D-glucose. Hydrolysis of sucrose is effectively catalyzed by acids and by enzymes called invertases, named in this case from the inversion in optical rotation. The hydrolysis mixture of D-glucose and D-fructose is called invert sugar for the same reason.

Maltose

This disaccharide occurs in small amounts in cell sap and to a greater degree in germinating and malted grains. In these cases the sugar is formed by partial hydrolysis of starch occurring in the presence of another group of enzymes called amylases. Maltose is a reducing sugar displaying reducing power equal to approximately half its weight of glucose. Presumably, then, maltose has one acetal and one semiacetal or free carbonyl group. Hydrolysis of maltose yields only glucose, the molecular ratio being one maltose to two glucose. Chemical studies of the linkage involved in coupling the two glucoses reveal the following structure for α-maltose:



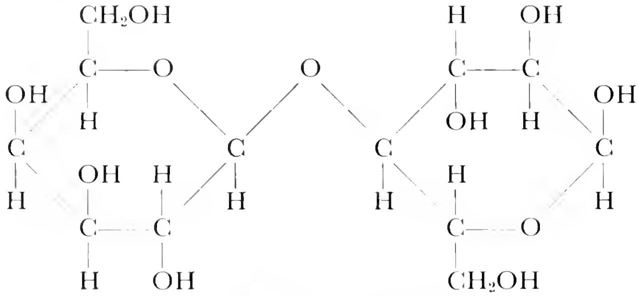
1-α-D-glucosido-4-α-D-glucose

With a free semiacetal structure, maltose shows the mutarotation characteristic of most reducing sugars and exists in solution in two equilibrium forms with perhaps a trace of intermediate having only the left-hand ring closed, the other opening and closing reversibly to yield the α and β forms.

Lactose

This carbohydrate, also called milk sugar, occurs in the milk of mammals to the extent of about 6 per cent in human milk and 4.8 per cent in cow's milk. Glucose is transported in the blood to the mammary gland, and lactose is synthesized there from the glucose. Since lactose is a disaccharide of both galactose and glucose, the galactose presumably must be formed by an inversion of the configuration of carbon atom four of glucose.

Lactose is a reducing sugar and exhibits mutarotation. It is less soluble and much less sweet than sucrose. The α diastereoisomer is less soluble than the β form. Sometimes the α isomer crystallizes in ice cream, making the product seem sandy in texture. The more soluble β isomer is used in diets for infants. α-Lactose may be represented by the following:



1-β-D-galactosido-1-α-D-glucose

Pentosans

These polysaccharides are made up of pentose units. Their structures are largely unknown, but two general types are recognized. Xylans yielding D-xylose on hydrolysis occur in vegetables, leaves, lichens, straw, corn cobs, oat hulls, wood. Some waste materials in this category are now employed industrially where the xylans are converted to furfural for use in organic synthesis and in oil refining. Arabans are found in the gummy exudates of trees and shrubs like cherry and mesquite. In fact these carbohydrates are found in many plant gums and juices and yield L-arabinose on hydrolysis.

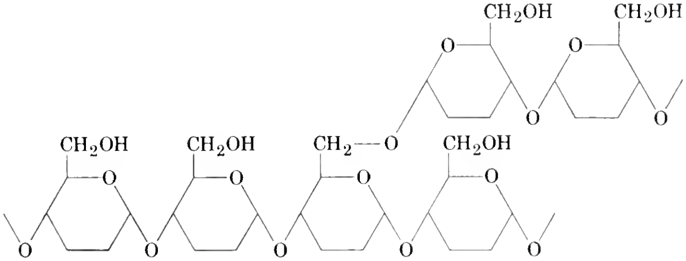
Starch

Hexosans are the polysaccharides made up of hexose units and are widespread in both plant and animal kingdoms. All living forms probably possess one or more kinds. Since the group is important, it will be discussed in terms of certain members.

Starch is the major reserve substance of higher plants and is produced during photosynthesis. It is stored throughout the plant and occasionally concentrated in roots, tubers, and seeds. Starch often occurs in grains whose size and appearance are characteristic of the plant source. These grains may burst on heating to yield a colloidal solution or gel, depending on the concentration. The starch molecules themselves are large and of two types differing enough so that starch may be fractionated. One fraction called amylose is composed of several hundred α-D-glucose subunits connected at the one and four positions to form a long thread-like molecule without branches. Amylose is readily dispersed in water and gives a typical blue color with iodine but does not form the characteristic gel or starch paste.

The other fraction of starch is amylopectin, which gives a purplish color with iodine and is difficult to disperse in water. However, when dispersed in sufficient concentrations, the amylopectin forms the typical starch gel. As in amylose, the compound is made up only of glucose with most of the linkages connecting one and four positions. However, amylopectin has a branched structure with the branches attached through 1–6 links. The naturally occurring molecules seem to differ in size but range up to perhaps 50,000 glucose subunits.

Both amylose and amylopectin are hydrolyzed in acid solution to D-glucose. If hydrolysis is interrupted early, large fragments are formed called dextrins. These materials possess adhesive properties and are used as adhesives on paper products. Starch breaks down into dextrins on heating, especially in the presence of moisture. The resulting dextrins then confer stiffness to clothes that have been starched and ironed. Enzymes called amylases catalyze the hydrolysis of starch to high yields of maltose, revealing the structural similarity between these two carbohydrates. The structure of amylopectin is represented below in schematic fashion with the ends of the structure shown connected to systems of the same type. Amylose resembles amylopectin in all respects except for the branches which are missing. Hence, amy-



amylopectin

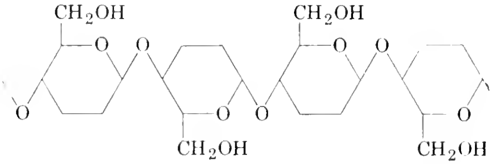
lose may be represented by a long chain of glucose rings connected as shown for its associated carbohydrate. The hexagons represent the usual configurations of the first five carbons of the α-D-glucose subunits.

Glycogen

Animal starch is the principal reserve carbohydrate of animals and is most abundant in muscle and liver. The same or a very similar carbohydrate occurs in yeast and certain fungi. Glycogen resembles amylopectin rather closely in both physical and chemical properties but is not identical with it. Similar linkages involving α-D-glucose occur in both carbohydrates, although the number and length of the branches may differ somewhat. The schematic structure of glycogen would be like that of amylopectin shown above.

Cellulose

This polysaccharide is also based on glucose, and the linkages are 1–4. However, the glucose is in the β form as chains of glucose units. Thus the structure of cellulose might be indicated by



representing a chain of great length. These rather linear arrangements are packed together to form the fibers of cotton and to serve as the structural materials in plants.

Besides carbon dioxide (free and in solution), mineral carbonates, coal, and perhaps petroleum, cellulose is probably the most abundant organic material. In fact, it is estimated that the quantity of carbon dioxide fixed as cellulose is about half the weight of this gas in the atmosphere. From this relationship it is clear that cellulose must be steadily degraded by bacteria and fungi, and the carbon dioxide returned to the atmosphere in order to maintain the carbon cycle.

Cellulose is resistant to the amylases attacking starch. The presence of the β form of glucose introduces a different enzyme specificity, and animals ordinarily do not possess enzymes capable of breaking cellulose into soluble fragments. However, the bacterial flora of certain animals do hydrolyze part of the cellulose, allowing the host to utilize it. The ruminants in particular gain an appreciable part of their energy from cellulose in this way. In most animals, the undigested cellulose supplies needed bulk to the digestive tract and aids in the elimination of food residues.

In woody tissues cellulose is associated with another material of great molecular size called lignin. Dried wood is about 65 per cent cellulose, 30 per cent lignin, and 1 to 2 per cent pentosans. In the manufacture of paper, wood is cooked with water, lime, and sulfur dioxide to remove the lignin, leaving the cellulose. These fibers are then spread into mats and rolled into sheets which may be treated in various ways for waterproofing or coated to provide a smooth writing surface. When certain grades of heavy paper are treated with sulfuric acid, translucent parchment paper is obtained.

Cellulose is insoluble in most solvents but does dissolve in solutions of cupric ammonium hydroxide or zinc chloride. Cotton treated with alkali becomes somewhat translucent, acquires a silky luster, and is called mercerized cotton. Rayon is made from cellulose by dissolving the material in sodium hydroxide and carbon disulfide. This viscous material is forced through small jets into an acidic solution of sodium bisulfite, regenerating the cellulose in small threads.

Esters of cellulose and nitric acid form in the presence of sulfuric acid. These nitrates are used in explosives, lacquers, celluloid, and collodion. Much of the former market for cellulose nitrates has been captured by cellulose acetate, much less flammable and widely used in lacquers, photographic film, and packaging materials. Celluloid has been replaced by various newer plastics which possess a greater diversity of properties and do not burn so readily.

Another group of plant materials called hemicelluloses is somewhat like ordinary cellulose. These substances may serve a structural function in some species and also be available as reserve carbohydrates for metabolic needs. Hydrolysis yields glucose, galactose, mannose, and glucuronic acid, although all may not and at least in some cases do not occur in the same hemicellulose. Little is known of linkages between the monosaccharide units.

Inulin

This polysaccharide serves as the carbohydrate reserve in a number of plants, dahlia and artichoke for example. Inulin forms a colloidal suspension in hot water, is non-reducing, and consists entirely of D-fructose units in the furanose form. It might be inferred, then, that the linkages probably are 2–6. Certain bacteria produce polysaccharides based on D-fructose, although the products apparently are not identical to inulin.

Amino Sugars

The chemistry and biochemistry of this group of compounds are under intensive investigation and much is being learned. Undoubtedly many amino sugars will be discovered and their biological roles worked out. Two are now known to be very widely distributed, especially in the animal kingdom. These are typical examples with a hydroxyl group replaced by an amino group. In both, the amino group is on carbon two, and the configuration is the same as for the parent sugar. D-Glucosamine is a reducing compound and shows mutarotation. It is found in bacteria and in polymeric form as chitin with the amino groups acetylated. Chitin is the principal component of the exoskeletons of insects and crustaceans.

D-Glucosamine, sometimes called chitosamine, also occurs in mucoitin sulfuric acid, a macromolecule made up of alternating units of glucuronic acid and glucosamine linked by 1–4 bonds. Some of the amino groups are acetylated; others exist as sulfamic acids, —NH—SO_3H. Mucoitin sulfuric acid is associated with proteins in saliva and other mucous secretions, and one form, heparin, is used to prevent the clotting of blood. Acetyl glucosamine is one of the constituents of hyaluronic acid. This acidic substance also contains glucuronic acid and is a major component of certain viscous fluids, such as the vitreous humor of the eye, synovial fluid of the joints, the jelly surrounding the ovum, and the umbilical cord.

The other abundant amino sugar is D-galactosamine, also called chondrosamine. It is found in certain bacterial polysaccharides but is best known for its occurrence in chondroitin sulfuric acid, where it appears to alternate with glucuronic acid with 1–4 linkages analogous to the structure of mucoitin sulfuric acid. Small amounts of chondroitin sulfuric acid appear in the mucous secretions. The material is especially plentiful in skin and cartilage, from which it is readily isolated.

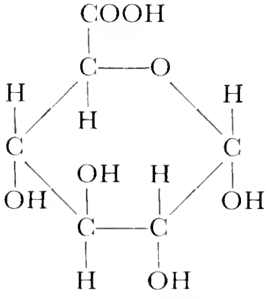
RELATED SUBSTANCES

A number of materials related to or containing carbohydrates have already been discussed briefly. Among these are chondroitin and mucoitin sulfuric acids, hyaluronic acid, and chitin. Other groups of compounds are sufficiently widespread and biologically important to warrant mention.

Derived Acids

The oxidation of the aldehyde group of the aldoses yields polyhydroxycarboxylic acids, called by the general name glyconic acids. Gluconic acid is the specific compound of this type resulting from D-glucose and is formed by certain bacteria. A derivative, phosphogluconic acid, is a key metabolic intermediate in a number of species. Other acids of this type have been synthesized in the laboratory, but nothing is known of the biology of these substances.

The glycuronic acids include the compounds with the other end of the molecule oxidized. Glucuronic acid, for example, has the structure shown and as expected exhibits mutarotation and reducing properties.



glucuronic acid

With the additional functional group, new types of reactions are possible. This acid has been mentioned as a component of mucoitin and chondroitin sulfuric acids and hemicellulose. It occurs in various gums like gum arabic and a variety of polysaccharides. In addition, animals and man synthesize glucuronic acid and esterify it with a number of toxic products which can then be excreted harmlessly in the urine. The body thus disposes of phenol, cresol, indoxyl, and similar otherwise dangerous hydroxy compounds.

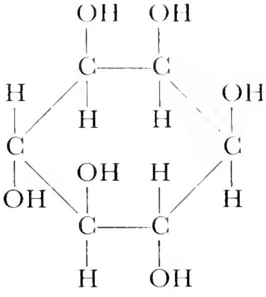
The related galacturonic acid also is found in polysaccharides but is best known from pectin, the gel-forming material of jams and jellies. Pectin is a polymer of the methyl ester of galacturonic acid with the units linked at the 1,4 positions. Alkaline solutions split the ester groups, yielding methanol and the salt of pectinic acid, the macromolecule still with the basic structure of pectin itself. Pectin is usually accompanied by polysaccharides made up of L-arabinose and D-galactose and is associated with them in practical usage.

Dicarboxylic acids called saccharic acids are formed when both ends of aldoses are oxidized. Little is known of the biochemistry of these compounds.

Alcohols

Reduction of the semiacetal group yields the corresponding polyalcohols. The most important are sorbitol, dulcitol, mannitol, and ribitol, corresponding to glucose, galactose, mannose, and ribose respectively. The first three occur in small amounts in plants, and ribitol is one of the components of the vitamin riboflavin.

A different type of polyalcohol, represented by inositol, is not derived directly from a sugar but is a derivative of cyclohexane. Inositol possesses chemical properties similar to those of sorbitol and the others and is included here for that reason. The structure is as follows:



inositol

A number of isomers are possible, the one shown being *meso*-inositol. It occurs in the phospholipides of the nervous systems of higher animals and is an essential growth factor for certain molds apparently incapable of synthesizing it.

Glycosides

This name is the generic term for the acetal derivatives of monosaccharides. According to this definition, the disaccharides and higher carbohydrates are glycosides. However, since these members of the class have already been considered, only the examples based on other alcohols than the hydroxyl groups of carbohydrates will be mentioned here.

This group of compounds is exceedingly varied and widespread, including many compounds that will be mentioned later in various connections. Many of the alcohols involved are polyfunctional. The pink and blue pigments of most blossoms and a few yellow colors are glycosides. These plant pigments are discussed on page 197. Another complex group includes the acetals of steroids. Of these compounds many are either animal hormones or plant products with pronounced pharmacological effects on animals. One class of natural detergents, the saponins, are glycosides, as are a wide variety of other plant materials, including acetals of various simple alcohols and phenols. As an example salicin from willow bark, the acetal of glucose and *o*-hydroxybenzyl alcohol, may be cited.

Among the more common carbohydrates occurring in these different groups of acetals are glucose, galactose, and fructose. In some glycosides the linkage is α; in others, β. For purposes of classification, the glycosides are sometimes divided according to the sugar involved, with the names glucoside, galactoside, and fructoside indicating the nature of the sugar.

Mucilages

The term *mucilage* is used to cover the colloidal materials of complex nature from plants that are capable of forming gels or have adhesive properties. Some of the materials described earlier, amylopectin for instance, might be so classified, but in the present case the word complex implies the presence of subunits other than monosaccharides alone. One of the best-known mucilages, agar, is a polymer of a sulfuric acid ester of galactose. Irish moss is a related member of this group. All appear to be sulfuric acid esters based on polysaccharides of galactose, mannose, and rhamnose, the last a methyl pentose.

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Lipides

4

When living tissues are treated with ether, alcohol, petroleum ether, or chloroform, a number of substances are extracted, among which is a group of compounds called lipides. Although the various members of this group are quite often chemically unrelated, they do have the following properties in common: (1) they are all appreciably soluble in one or more of the solvents mentioned above, whereas most of them are relatively insoluble in water; (2) they all exist as actual or potential esters of fatty acids; (3) they can be utilized by a living organism.

When lipides are classified, they are usually divided into three main groups, namely, simple lipides, compound lipides, and hydrolytic products of lipides. Simple lipides are defined as organic esters which, upon hydrolysis, yield only aliphatic alcohols and aliphatic monocarboxylic acids. Included in this category are the fats and waxes. Upon hydrolysis, compound lipides yield aliphatic alcohols, aliphatic monocarboxylic acids, and another product, such as a carbohydrate, phosphoric acid, or a nitrogenous base. Examples of this group are the phospholipides and the glycolipides. The hydrolytic products of lipides include the fatty acids, various alcohols, such as glycerol and the sterols, and a number of nitrogenous compounds such as choline or sphingosine.

FATTY ACIDS

Occurrence

Fatty acids are integral constituents of every simple and compound lipide. In the majority of instances they are found in nature as esters when combined with an alcohol. Occasionally free fatty acids exist in immature seeds. By and large, fatty acids are straight-chain aliphatic monocarboxylic acids, although in recent years a few branched-chain acids have been found. Most of the members of this series contain an even number of carbons in the molecule. Thus, the first member of the series, butyric acid, contains four carbons; the second member, caproic acid, contains six carbons in the molecule. Although the low-molecular-weight members of the series are found occasionally in natural products, they are not nearly so abundant as higher-molecular-weight acids such as palmitic acid ($C_{15}H_{31}COOH$), stearic acid ($C_{17}H_{35}COOH$), and oleic acid ($C_{17}H_{33}COOH$).

Classification

Fatty acids may be divided into four classes according to their molecular structure. There are saturated, unsaturated, hydroxy, and cyclic acids (see classification in Table 4–1). Of these, the saturated and unsaturated acids are the most common in natural products.

The first three members of the saturated fatty acid series, butyric, caproic, and caprylic acids, are liquid at 20°C., while the fourth member, capric acid, is a semisolid at this temperature. These four acids are volatile with steam and are referred to as the volatile fatty acids. This property of volatility is utilized in testing for the presence of these acids in such natural products as butterfat.

The volatile fatty acids are easily recognized by their odor. Butyric acid has a strong, penetrating odor quite suggestive of rancid butter. Caproic, caprylic, and capric acids are powerfully reminiscent of a goat and are, in fact, referred to as the goat acids.

Saturated fatty acids with more than ten carbons in the molecule are not volatile with steam, are odorless, and have melting points well above 20°C. The most abundant saturated acids occurring in the lipides are the sixteen- and eighteen-carbon members, palmitic and stearic acids. The hard consistency of many fats may be attributed to the presence of large percentages of these relatively high-melting components esterified with glycerol.

When a fatty acid has one or more double bonds in the molecule, it is said to be unsaturated. Generally speaking, the unsaturated fatty acids are of high molecular weight, having twelve or more carbons in the molecule. The predominant members of this class have eighteen carbons, for example, oleic acid (one double bond), linolic acid (two double bonds), and linolenic acid (three double bonds).

The presence of one or more double bonds in the fatty acid confers quite different properties on the molecule from those evident in a

TABLE 4–1. Classification of Fatty Acids Present as Glycerides in Fats

Name		Structural Formula
Common	Systematic	
	I. Saturated Fatty Acids	
A. Straight-chain Series		
Butyric	Butanoic	C_3H_7COOH
Caproic	Hexanoic	$C_5H_{11}COOH$
Caprylic	Octanoic	$C_7H_{15}COOH$
Capric	Decanoic	$C_9H_{19}COOH$
Lauric	Dodecanoic	$C_{11}H_{23}COOH$
Myristic	Tetradecanoic	$C_{13}H_{27}COOH$
Palmitic	Hexadecanoic	$C_{15}H_{31}COOH$
Stearic	Octadecanoic	$C_{17}H_{35}COOH$
Arachidic	Eicosanoic	$C_{19}H_{39}COOH$
Behenic	Docosanoic	$C_{21}H_{13}COOH$
Lignoceric	Tetracosanoic	$C_{23}H_{47}COOH$
B. Branched-chain Series		
Isobutyric	2-Methylpropanoic	C_3H_7COOH
Isovaleric	3-Methylbutanoic	C_4H_9COOH
Tuberculostearic	10-Methyloctadecanoic	$C_{17}H_{35}COOH$
	II. Unsaturated Fatty Acids	
A. Oleic Acid Series		
1. Straight-chain		
Lauroleic	3-Dodecenoic	$C_{11}H_{21}COOH$
Myristoleic	9-Tetradecenoic	$C_{13}H_{25}COOH$
Myristoleic	5-Tetradecenoic	$C_{13}H_{25}COOH$
Palmitoleic	9-Hexadecenoic	$C_{15}H_{29}COOH$
Oleic	9-Octadecenoic	$C_{17}H_{33}COOH$
Petroselinic	6-Octadecenoic	$C_{17}H_{33}COOH$
Vaccenic	11-Octadecenoic	$C_{17}H_{33}COOH$
Hepatic oleic	12-Octadecenoic	$C_{17}H_{33}COOH$
Gadoleic	9-Eicosenoic	$C_{19}H_{37}COOH$
Cetoleic	11-Docosenoic	$C_{21}H_{41}COOH$
Erucic	13-Docosenoic	$C_{21}H_{41}COOH$
Selacholeic	15-Tetracosenoic	$C_{23}H_{45}COOH$
2. Branched-chain		
Tiglic	2-Methyl-2-butenoic	C_4H_7COOH
B. Linolic Acid Series		
1. Double bond		
Linolic	9,12-Octadecadienoic	$C_{17}H_{31}COOH$
2. Triple bond		
Tariric	6-Octadecynoic	$C_{17}H_{31}COOH$

TABLE 4–1. (Continued)

Name		Structural Formula
Common	Systematic	
C. Linolenic Acid Series		
Linolenic	9,12,15-Octadecatrienoic	$C_{17}H_{29}COOH$
Eleostearic	9,11,13-Octadecatrienoic	$C_{17}H_{29}COOH$
D. Arachidonic Series		
Parinaric	9,11,13,15-Octadecatetraenoic	$C_{17}H_{27}COOH$
Arachidonic	5,8,11,14-Eicosatetraenoic	$C_{19}H_{31}COOH$
E. Clupanodonic Series		
Clupanodonic	4,8,12,15,19-Docosapentaenoic	$C_{21}H_{33}COOH$
III. Unsaturated Monohydroxy Fatty Acids—Ricinoleic Series		
Ricinoleic	12-Hydroxy-9-octadecenoic	$C_{17}H_{32}OHCOOH$
IV. Cyclic Fatty Acids—Chaulmoogric Series		
Hydnocarpic	11-Δ^2-Cyclopentenyl-*n*-undecanoic acid	$C_{15}H_{27}COOH$
Chaulmoogric	13-Δ^2-Cyclopentenyl-*n*-tridecanoic acid	$C_{17}H_{31}COOH$

saturated fatty acid. One of the outstanding effects is on the melting point of the acid. Whereas stearic acid melts at 69.6°C., oleic acid melts at 13°C. Linolic and linolenic acids melt at −5°C. and −14.5°C., respectively. Thus, the introduction of one or more double bonds in a long-chain fatty acid produces a compound which is liquid at 20°C. The liquid state of most vegetable fats can be attributed to large percentages of unsaturated fatty acid components.

Unsaturated fatty acids are far more chemically reactive than their saturated analogues. For example, oxygen reacts with the double bond of an unsaturated acid to form an ethylene oxide linkage. In the case of a polyunsaturated acid, such as linolenic, the addition of oxygen to the double bonds results in the production of a resinous compound. Fats containing large quantities of such polyunsaturated acids are important to the paint and varnish industry, where the formation of a tough resinous film is desirable in the drying of these products. Linseed and tung fats are examples of natural products used in the paint industry.

Reactions

Although fatty acids enter into all the reactions customarily expected of the funtional groups which they contain, few of these merit discussion in this book. Those that do are hydrogenation and reduction, soap production, and esterification.

Unsaturated fatty acids react quantitatively with hydrogen to produce the corresponding saturated analogs. Thus 1 mole of oleic, linolic, and linolenic acid react with 1, 2, and 3 moles of hydrogen, respectively, to form stearic acid. By a similar reaction, these same unsaturated acids react with a halogen, such as bromine, to produce di-, tetra-, and hexabromostearic acids. Considerable use has been made of these bromo derivatives in the separation and identification of mixtures of the unsaturated acids. The free acids can be regenerated from the bromo derivatives by the action of zinc.

Under more stringent conditions of temperature and pressure and in the presence of a catalyst, the carboxyl group of any fatty acid may be reduced to yield an alcohol. This reaction has proved to be of great commercial significance in the preparation of a large number of soap substitutes called detergents. The alcohol formed by the reduction of a higher-molecular-weight fatty acid is treated with sulfuric acid to form the sulfuric acid ester, which is then converted to the sodium salt, as shown in the following equations:

$$\underset{\text{lauryl alcohol}}{CH_3(CH_2)_{10}CH_2OH} + H_2SO_4 \rightarrow \underset{\text{lauryl sulfate}}{CH_3(CH_2)_{10}CH_2OSO_3H} + H_2O$$

$$\underset{\text{lauryl sulfate}}{CH_3(CH_2)_{10}CH_2OSO_3H} + NaOH \rightarrow \underset{\text{sodium lauryl sulfate}}{CH_3(CH_2)_{10}CH_2OSO_3Na} + H_2O$$

The final product is a water-soluble substance with all the desirable characteristics of a soap. These sulfated alcohols are especially desirable in hard-water areas because their calcium and magnesium salts are water soluble, thus eliminating the bothersome precipitation of the insoluble salts formed when true soaps are used.

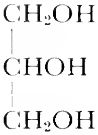
Fatty acids form salts with inorganic bases in a typical neutralization reaction, as shown by the following equation:

$$\underset{\text{palmitic acid}}{CH_3(CH_2)_{14}COOH} + NaOH \rightarrow \underset{\text{sodium palmitate}}{CH_3(CH_2)_{14}COONa} + H_2O$$

Needless to say, a large variety of bases and fatty acids can be used to produce a myriad of soaps with different properties and applications. Some of these will be considered in a later section of this chapter.

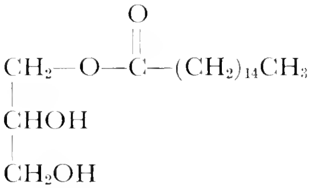
By far the most important reaction of fatty acids from a biological standpoint is that of ester formation. In living tissues fatty acid derivatives can and do react with a variety of alcohols to form biologically important esters. Thus, a fatty acid might react with a high-molecular-weight monohydric alcohol, such as cetyl alcohol [$CH_3(CH_2)_{11}CH_2OH$], to form a wax. On the other hand, a fatty acid derivative might react with a carbohydrate and a nitrogenous base to form a glycolipide. The most abundant of all esters formed in living tissues, however, is the reaction product of these activated fatty acids with the trihydric alcohol, glycerol. The resulting products, glycerides, make up the hundreds of different animal and vegetable fats.

Since glycerol has three hydroxyl groups (see formula below) it is possible to have one, two, or three ester linkages in the same glyceride molecule. In other words, it is possible to have mono-, di-, or triglycerides, examples of which are shown below.



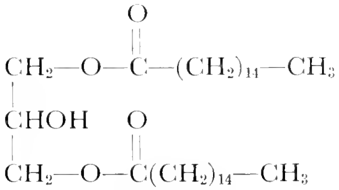
$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

glycerol



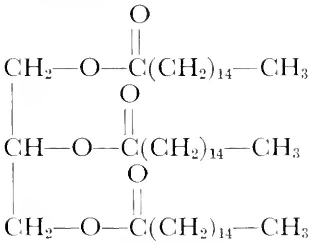
$$\begin{array}{l} CH_2{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}(CH_2)_{14}CH_3 \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

1-monopalmitin
(a monoglyceride)



$$\begin{array}{l} CH_2{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}(CH_2)_{14}{-}CH_3 \\ | \\ CHOH \\ | \\ CH_2{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_{14}{-}CH_3 \end{array}$$

1,3-dipalmitin
(a diglyceride)

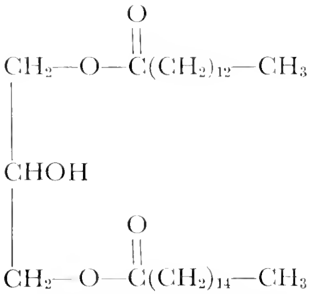


$$\begin{array}{l} CH_2{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_{14}{-}CH_3 \\ | \\ CH{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_{14}{-}CH_3 \\ | \\ CH_2{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_{14}{-}CH_3 \end{array}$$

1,2,3-tripalmitin
(a triglyceride)

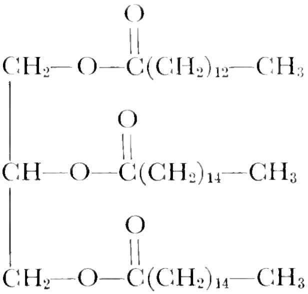
As can be seen from the example, there is a structural isomer of 1-monopalmitin, namely, 2-monopalmitin. These isomeric forms have different physical properties such as melting point, boiling point, viscosity, and density. Since it is possible that either or both isomers might be present in a naturally occurring fat, the consistency of the fat can be affected materially by the amount and type of isomer present. In a similar fashion the two isomeric forms of dipalmitin, if present in a fat, will contribute to its overall physical form.

Thus far we have considered the glyceride forms in which one particular fatty acid has combined with one, two, or all three hydroxyl groups of glycerol. Such compounds are called simple glycerides. If two or three different fatty acids are combined with a glycerol molecule, the compound is called a mixed glyceride. Examples of mixed glycerides are shown on page 80.



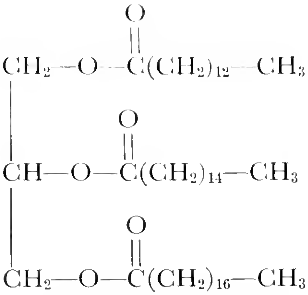
$$\begin{array}{l} \mathrm{CH_2-O-\overset{O}{\overset{\|}{C}}(CH_2)_{12}-CH_3} \\ | \\ \mathrm{CHOH} \\ | \\ \mathrm{CH_2-O-\overset{O}{\overset{\|}{C}}(CH_2)_{14}-CH_3} \end{array}$$

1-myristo-3-palmitin



$$\begin{array}{l} \mathrm{CH_2-O-\overset{O}{\overset{\|}{C}}(CH_2)_{12}-CH_3} \\ | \\ \mathrm{CH-O-\overset{O}{\overset{\|}{C}}(CH_2)_{14}-CH_3} \\ | \\ \mathrm{CH_2-O-\overset{O}{\overset{\|}{C}}(CH_2)_{14}-CH_3} \end{array}$$

1-myristo-2,3-dipalmitin



$$\begin{array}{l} \mathrm{CH_2-O-\overset{O}{\overset{\|}{C}}(CH_2)_{12}-CH_3} \\ | \\ \mathrm{CH-O-\overset{O}{\overset{\|}{C}}(CH_2)_{14}-CH_3} \\ | \\ \mathrm{CH_2-O-\overset{O}{\overset{\|}{C}}(CH_2)_{16}-CH_3} \end{array}$$

1-myristo-2-palmito-3-stearin

Mixed glycerides also exist in several isomeric forms. A mixed diglyceride has three geometrically isomeric forms. A triglyceride such as 1-myristo-2,3-dipalmitin may occur in two isomeric forms, while a triglyceride containing three different fatty acid residues can be written structurally in three different ways.

On the basis of our discussion of the various isomeric forms of glycerides, one can predict that, if glycerol reacted randomly with palmitic acid, 5 different glycerides (2 mono-, 2 di-, and 1 triglyceride) could possibly be formed. Again, if glycerol reacted randomly with palmitic and myristic acids, 17 different products might be formed. Finally, if 3 different acids were esterified with glycerol in such a way that all possible glycerides were produced, 39 products would be included in the mixture. Thus an animal or vegetable fat which yields 4, 5, or perhaps 6 or more different fatty acids on hydrolysis must be composed of an impressive number of different types and isomeric forms of glycerides. This fact, in itself, accounts for the relatively slow progress made in elucidating the precise chemical structure of any one fat.

FATS

Occurrence

From the standpoint of natural abundance and commercial value, fats are the most important of all the groups included in a lipide classification. These simple lipides are found in virtually every living tissue of plant or animal origin. They are stored in greatest quantity, however, in the fat depots of the living organism. For example, in an animal we find that fat reservoirs form beneath the skin, in the abdomen and around the intestines, and surrounding such organs as the heart, liver, and kidneys. Although small quantities of fat may be deposited throughout a plant, the greatest concentration of reserve lipide is located in the seed or fruit.

Although heredity plays a very dominant role in the type and amount of fat deposited in the various tissues of plants and animals, environment also affects lipide deposition. For example, although flax may be grown in climates ranging from fairly warm to cold, the linseed fat (oil) obtained from the flaxseed of plants grown in one climate will differ materially in both physical and chemical properties from that of plants grown in another climate. In general, it can be stated that the colder the climate, the greater will be the amount of polyunsaturated acids found in the glyceride fraction.

The activity of an animal largely governs the type of fat laid down in the carcass. A very active animal deposits the harder saturated fats in the tissues, whereas a sedentary member of the same species tends to deposit the softer, more unsaturated fats.

Diet also affects the nature of the carcass fat deposited by an animal. For example, a hog fed on large quantities of peanut or cottonseed meal deposits large quantities of unsaturated glycerides. These low-melting glycerides, in turn, produce an undesirably oily carcass. Such pork is referred to as soft pork.

Physical Properties

As explained in a previous section, fats may be either solids or liquids at 20°C. Most of the common animal fats are solid at this temperature, whereas the majority of vegetable fats (oils) are liquid at the same temperature. In fact this solidity or liquidity at 20°C. has served in the past as an arbitrary means of classification of these simple lipides into animal fats (solids) and vegetable oils (liquids). In many instances such a classification tends to be misleading, since some vegetable oils are solid at this temperature while some animal fats are semisolid or even liquid at 20°C. Moreover such a classification overlooks entirely the marine and bacterial lipides which, depending upon their origin, may be solid or liquid at 20°C.

Fats are lighter than water and usually have a density between 0.8 and 0.9 g./cm.^{3} The fact that they are poor conductors of heat makes them particularly useful as body insulators. When pure, fats are colorless, virtually odorless, and possess an extremely bland taste. They are capable of easily absorbing a variety of odors and hence flavors during storage. For example, the young housewife soon discovers that the flavor of an onion will quickly permeate butter that is stored with the tuber in a refrigerator. In some instances this characteristic absorption of odor and flavor is used to advantage. For example, the delicate perfumes of some flowers can be isolated by placing the petals in contact with fat for a period of time, then extracting the fat with cold alcohol and concentrating the essence.

Structure and Chemical Properties

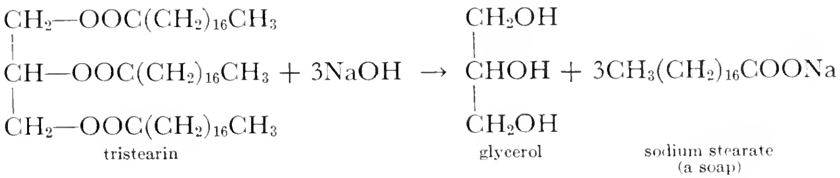
Fats are complex mixtures of glycerides. The degree of complexity of the mixture depends on the number of different fatty acids and the amount of each acid available for the formation of glycerides. In addition, the difference in the nature of the fats produced by different organs of a plant or animal suggests that such organs enjoy a preference for certain fatty acids and hence for certain glyceride mixtures.

The principal difference between animal and vegetable fats lies in the amount and type of unsaturated fatty acids in the glycerides. Both animal and vegetable fats contain a considerable amount of oleic acid, but only vegetable fats can be considered rich in the polyunsaturated acids, linolic and linolenic. The presence of appreciable quantities of these unsaturated acids in the glycerides of vegetable fats accounts for their liquidity at 20°C. On the other hand, the predominance of saturated glycerides in animal fats explains their solid state at room temperature.

Although a number of saturated fatty acids may be isolated from vegetable fats, palmitic acid predominates. In animal fats a larger variety of saturated acids may be found although stearic acid proves to be the most abundant. In any case, it is easy to understand that variations in the number of fatty acids, in the type of fatty acids, and in the amounts of each acid will result in the formation of structurally different fats, each possessing its own characteristic physical and chemical properties.

Since fats are organic esters, they are easily hydrolyzed. Hydrolysis or splitting of fats into their constituent parts may be accomplished by various means. For example, fats hydrolyze very slowly when intimately mixed with water. On the other hand, hydrolysis becomes fairly rapid when a fat is treated with steam. The rate of hydrolysis is increased even more if an emulsifying agent is added, thereby increasing the contact between the fat and water surfaces. An example of such an emulsifier is Twitchell's reagent, which consists of naphthalene, oleic acid, and sulfuric acid. When a fat that has been preheated with steam comes into contact with small quantities of Twitchell's reagent, and the mixture is heated to the boiling point, hydrolysis occurs in a very short time.

When fats are intimately mixed with an alcoholic solution of a strong alkali, such as sodium or potassium hydroxide, and are heated, the glyceride molecules are split, yielding glycerol and the sodium or potassium salts of the fatty acids. This process is called saponification (soap producing), and the metallic salts of the fatty acids are called soaps. The saponification reaction may be represented as follows:



$$\underset{\text{tristearin}}{\begin{array}{l}CH_2{-}OOC(CH_2)_{16}CH_3\\ |\\ CH{-}OOC(CH_2)_{16}CH_3\\ |\\ CH_2{-}OOC(CH_2)_{16}CH_3\end{array}} + 3NaOH \rightarrow \underset{\text{glycerol}}{\begin{array}{l}CH_2OH\\ |\\ CHOH\\ |\\ CH_2OH\end{array}} + \underset{\substack{\text{sodium stearate}\\ \text{(a soap)}}}{3CH_3(CH_2)_{16}COONa}$$

Soaps may be divided into two classes, hard soaps and soft soaps. Hard soaps, such as the common bar soaps, are the sodium salts of the higher fatty acids. Soft soaps are the potassium salts of the higher fatty acids and are usually marketed as semiliquids or pastes. The fatty acid salts of magnesium and calcium and the heavy metals, such as lead and zinc, are insoluble in water. When a sodium or potassium soap is added to "hard" water (water containing calcium or magnesium salts in solution), the sodium or potassium atom is replaced by calcium or magnesium, forming a soap insoluble in water:

$$\underset{\substack{\text{soluble}\\ \text{sodium soap}}}{2CH_3(CH_2)_{16}COONa} + \underset{\substack{\text{calcium}\\ \text{bicarbonate}}}{Ca(HCO_3)_2} \rightarrow \underset{\substack{\text{insoluble}\\ \text{calcium soap}}}{[CH_3(CH_2)_{16}COO]_2Ca} + \underset{\substack{\text{sodium}\\ \text{bicarbonate}}}{2NaHCO_3}$$

The formation of such insoluble soaps explains the presence of "curds" when ordinary soaps are used in hard water.

Calcium soaps are employed industrially as lubricating greases. Zinc soaps, especially zinc stearate, are used extensively in talcum powders and cosmetics. In paints, lead, manganese, and cobalt soaps hasten the process of drying.

Fats containing unsaturated fatty acids can be hydrogenated to produce a fat containing only saturated glycerides. Thus, liquid fats can be converted to solids having appreciably higher melting points. It is also possible to partially hydrogenate a liquid fat to form a product having properties intermediate between those of the liquid and the solid fat. Commercial use is made of such controlled hydrogenation in the production of shortenings and margarines from vegetable fats.

Rancidity

Fats exposed to light, air, heat, or moisture for an extended period of time slowly develop obnoxious flavors and odors and are said to be rancid. There are two principal types of fat spoilage, namely, hydrolytic and oxidative rancidity.

Hydrolytic rancidity is the result of the hydrolysis of fats with the liberation of small amounts of one or more volatile fatty acids (butyric through capric). Dairy products are particularly affected by this type of spoilage because of the rather large amounts of the low-molecular-weight acids found in the glycerides of milk and milk products. The liberation of a trace of butyric acid, for example, will affect the odor and taste of dairy products to the point where sales value is appreciably lowered.

All fats undergo partial hydrolysis when exposed to moisture for an extended period of time. However, the majority of them do not become rancid, owing to the fact that they do not contain volatile fatty acids and therefore do not develop the obnoxious odors or flavors associated with rancidity.

From a fat-economy standpoint, oxidative rancidity is the more important form of spoilage. In this type, the unsaturated fatty acid fragments of the glycerides are oxidized at their double bonds with the ultimate production of aldehydes, ketones, and acids with fewer carbon atoms in the molecule. The objectionable odor and taste of an oxidized fat are due to the presence of these decomposition products. A saturated fat (that is, one without any unsaturated acids in the glycerides) is almost completely resistant to oxidation and therefore does not exhibit oxidative rancidity.

It has been known for a long time that different unsaturated fats, when exposed to an atmosphere of pure oxygen, do not become rancid in the same length of time. Indeed, certain unsaturated fats are quite resistant to oxidation. However, when this initial resistance is overcome, all unsaturated fats absorb large quantities of oxygen with considerable rapidity and develop, with equal rapidity, a characteristic rancid odor and flavor. The length of time involved before a fat or oil begins to absorb large quantities of oxygen is known as the induction period. The induction period of a fat is of vital importance in so far as predicting its keeping quality is concerned. Fats with short induction periods cannot be stored for any length of time and consequently cannot be used in products where long storage is necessary.

The length of the induction period of a fat or oil seems to depend largely on the presence of small concentrations of substances other than the fat itself. These substances may be divided into two main classes: (1) compounds which inhibit oxidation of the fat, and (2) compounds which accelerate decomposition. The first group (those which inhibit oxidation) includes compounds which are readily oxidized and which absorb large quantities of oxygen. A compound which prevents oxidation of a fat or oil and, in so doing, lengthens the induction period is called an antioxidant. Such a substance will continue to inhibit the oxidation of the material it is protecting until the antioxidant itself is completely oxidized. Although the chemical nature of many of the naturally occurring antioxidants is unknown, it has been definitely established that ascorbic acid and α-tocopherol (vitamins C and E) have effective antioxygenic properties. Many synthetic compounds have been shown to possess antioxygenic properties, but some of them cannot be used in food products because of their toxicity.

Opposing the action of the antioxidants in fats and oils is another group of compounds which accelerates the oxidation of the parent compound. Such substances are known as pro-oxidants; copper lactate is an example. Among the most noted pro-oxidants are the copper, iron, and nickel salts of organic acids. It is generally believed that the majority of pro-oxidants are formed during the processing and refining of a fat. Regardless of their origin, pro-oxidants are important factors when considering the keeping qualities of a fat.

Fat Analysis

It was mentioned in a previous paragraph that fats are mixtures of glycerides; even the simplest fats are complex mixtures of individual glyceride molecules. For example, if there are three different acids attached to the glycerol molecule, three different geometric isomers can occur, according to the position of attachment of these acids on the glycerol molecule. If there are four different acids in the mixture, twelve theoretical triglyceride isomers are possible. Likewise, if five acids are involved, there are thirty possible mixed triglycerides. Of course, in the foregoing discussion we have considered only isomeric triglycerides. One must also remember that there will be some isomeric mono- and diglycerides in the mixture. Such a situation seriously complicates the problem of analysis, since no effective methods are available for the isolation of one isomer from another. Consequently fat analysis depends upon the collection of certain available data which are more or less characteristic and constant for each type of fat. These determinations are called fat constants. The fat constants are physical and chemical measurements which seem to remain relatively constant for fats prepared properly from the same source.

Among the physical constants most commonly determined are refractive index, melting point, viscosity, and specific gravity. The melting point, although not an extremely accurate determination, is a guide in detecting adulteration of fats and also helps in approximating the amount of unsaturated fatty acids present. Often, instead of determining the melting point of a fat, the chemist will determine the solidification point of the free fatty acids. This is accomplished by saponifying the fat and treating the soaps with mineral acid, thus liberating the mixture of the free fatty acids which can be isolated and used to determine the solidification point.

Chemical constants most frequently used in the analysis of a fat include acid number, saponification number, iodine number, and Reichert-Meissl number. The type of information desired concerning the fat determines the number of these constants measured.

The acid number, defined as the number of milligrams of KOH required for the neutralization of 1 g. of fat, is useful as a measure of the free fatty acids in a fat. Obviously, a fat which has been exposed to moisture for an extended period of time might have an appreciable acid number, indicating that hydrolysis of glycerides has occurred. A fat which has been both processed and stored properly has a very low acid number.

The saponification number is defined as the number of milligrams of KOH required to saponify completely 1 g. of fat. This test is based on the fact that all fatty acids, regardless of molecular weight, are monocarboxylic, and therefore each acid molecule will unite with only one potassium ion in the formation of soap. If the fat is composed of acids of high molecular weight, such as palmitic, stearic, or oleic acid, there will be fewer molecules of fatty acids per gram of fat. Therefore, the number of milligrams of KOH required to saponify

1 g. of the fat will be relatively few, and the saponification number will be low. On the other hand, the saponification number will be high if there is a predominance of shorter-chain fatty acids combined with the glycerol in a fat. It can be stated, therefore, that the saponification number varies inversely with the mean molecular weight of the fatty acids bound as glycerides.

The degree of unsaturation of a fat may be determined by allowing a known amount of fat to react with solutions of known strength containing iodine chloride (Wijs solution) or iodine bromide (Hanus solution). The iodine adds to the double bonds of the unsaturated fatty acid portion of the glyceride molecules, each double bond absorbing 2 atoms of iodine. The iodine number is defined as the number of grams of iodine absorbed by 100 g. of a fat. Although the iodine number indicates the total amount of unsaturation in a fat, it gives no indication of the number of double bonds in any particular fatty acid or glyceride.

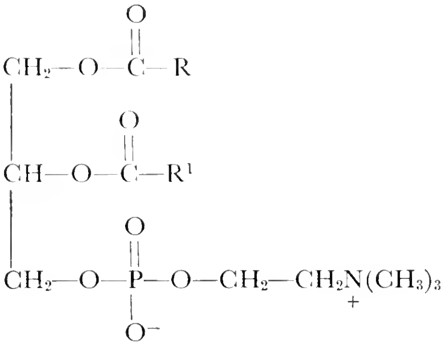
An index of the amount of volatile fatty acids in the glycerides of a fat can be ascertained by means of the Reichert-Meissl number. A known weight of fat is saponified, the soaps are converted to free fatty acids, and these are subjected to distillation with live steam. Those acids containing from 4 to 10 carbons are quite volatile with steam and are removed by distillation and titrated with 0.1*N* KOH. The number of milliliters of 0.1*N* KOH required to neutralize the volatile, soluble fatty acids from 5 g. of a fat is called the Reichert-Meissl number. Butterfat is an exception to the rule which states that naturally occurring fats contain relatively small amounts of volatile fatty acids. Whereas the Reichert-Meissl numbers of coconut oil and palm oil range between 5 and 8, the value for butterfat ranges from 21 to 33. The high Reichert-Meissl number of butterfat makes it possible to detect the presence of foreign fats which are sometimes used as adulterants in the manufacture of butter.

PHOSPHOLIPIDES

From a biological standpoint, the phospholipides are one of the most important classes of lipides. Although found in most plant and animal tissues, they are particularly abundant in such active organs as the liver and the brain. The best-known members of the phospholipide group are the lecithins, the cephalins, and the sphingomyelins.

Lecithins and cephalins are soluble in ether and are extracted from a tissue together with the fats and other lipides. They may be separated from the fats by treating the ether extract with acetone. The lecithins and the cephalins precipitate, while the other lipides will remain in solution. Sphingomyelins differ from the other phospholipides in that these compounds are soluble in neither acetone nor ether. Consequently, the sphingomyelins are not removed from a tissue by the customary ether extraction, but rather by extraction with hot alcohol. On cooling, the sphingomyelins crystallize from the alcohol as a white solid.

On complete hydrolysis, lecithins yield two molecules of fatty acids, glycerol, phosphoric acid, and the nitrogenous base, choline. At one time it was generally believed that every lecithin contained one saturated and one unsaturated fatty acid radical in the molecule. Recent investigation has shown that both acids may be saturated, or both unsaturated, or one saturated and one unsaturated. It is probable that a large number of lecithins exist and that the number is limited only by the various binary combinations of the fatty acids. The following formula is representative of a typical lecithin:

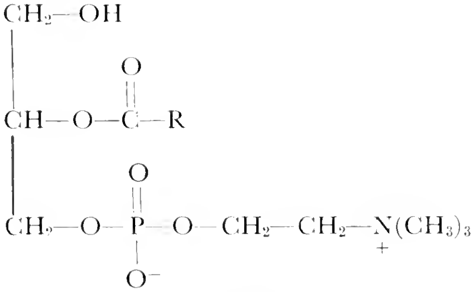


α-lecithin

The name of the above compound is derived from the fact that the phosphoric acid-choline group is attached to a terminal (α) carbon atom of the glycerol. If the phosphoric acid-choline group were attached to the central (β) carbon atom, the compound would be described as a β-lecithin. The naturally occurring lecithins that have been isolated thus far have been shown to be of the α variety. Although this indicates that α-lecithins are strongly predominant in nature, it does not completely exclude the possibility of the existence of β-lecithins.

Hydrolysis of lecithins can be accomplished in the laboratory by the use of moderately strong acids or bases to yield fatty acids, choline, and α-glycerophosphoric acid. The ester linkage existing between glycerol and phosphoric acid is exceptionally strong and can be broken only by prolonged boiling with a strong acid.

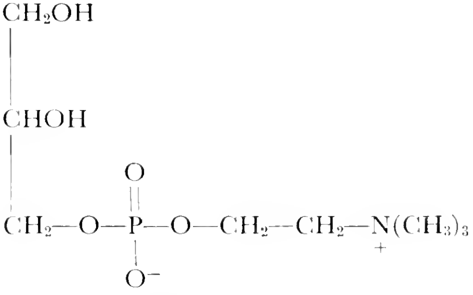
Lecithins are hydrolyzed in living tissues by means of certain enzymes called lecithinases. There are apparently a number of different lecithinases, each one capable of splitting the lecithin molecule at a different linkage. For example, lecithinase A removes one fatty acid residue and yields a product referred to as a lysolecithin. Certain snakes, such as the cobra, produce a venom containing lecithinase A. When the venom is injected into a victim, lysolecithin is produced and is poisonous owing to its ability to dissolve (hemolyze) red blood cells. Following is the structure for a typical α-lysolecithin:



$CH_2OH-CH(O-CO-R)-CH_2-O-PO(O^-)-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$

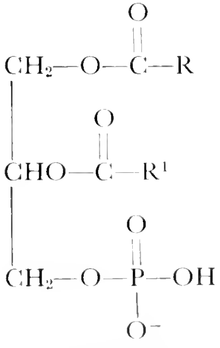
α-lysolecithin

Lecithinase B is responsible for the cleavage of both fatty acid residues from the lecithin molecule, thus producing α-glycerophosphoryl choline. Still another lecithinase is capable of removing the nitrogenous base, choline, from lecithin to form a compound known as a phosphatidic acid. Such phosphatidic acids usually exist in living tissues as metallic salts.



$CH_2OH-CHOH-CH_2-O-PO(O^-)-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$

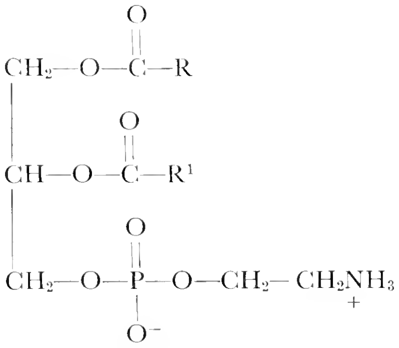
α-glycerophosphoryl choline



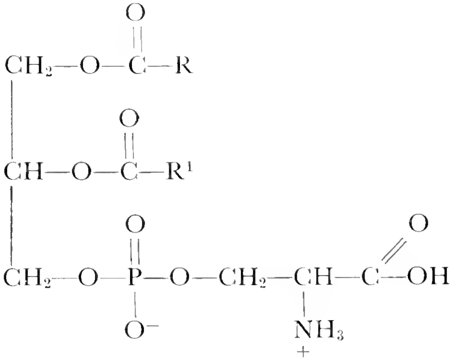
$CH_2(O-CO-R)-CH(O-CO-R^1)-CH_2-O-PO(O^-)-OH$

α-phosphatidic acid

Cephalins, which occur in many living tissues but are particularly abundant in brain, differ in structure from the lecithins only in the type of nitrogenous base bound to the phosphoric acid residue. Instead of choline, cephalins have been found to contain either ethanolamine ($HOCH_2CH_2NH_2$) or serine ($\mathrm{HO{-}CH_2{-}\underset{\underset{\displaystyle NH_3^+}{|}}{CH}{-}COO^-}$). In order to distinguish between the two types of cephalins the names phosphatidyl ethanolamine and phosphatidyl serine are used.



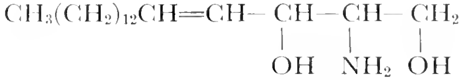
α-phosphatidyl ethanolamine



α-phosphatidyl serine

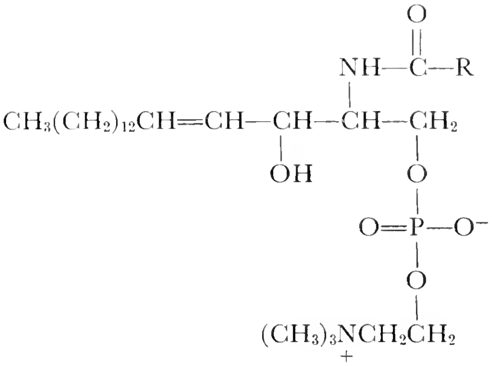
The two types of cephalins may be separated from each other by making use of the fact that the phosphatidyl ethanolamines are alcohol soluble whereas the phosphatidyl serines are alcohol insoluble.

The third group of phospholipides mentioned in a previous paragraph are the ether- and acetone-insoluble sphingomyelins. Although originally isolated from brain tissue, these compounds have been shown to occur in many organs of the body, as well as in blood and milk. On complete hydrolysis, sphingomyelins yield an amino alcohol (sphingosine), a fatty acid (usually lignoceric), phosphoric acid, and choline. It has been shown that the fatty acid is attached to the amino group of sphingosine to form an amide called lignoceryl sphingosine. This substance, which can be formed by the partial hydrolysis of sphingomyelin, is found in the spleen and liver of the pig.



$$\mathrm{CH_3(CH_2)_{12}CH{=}CH{-}\underset{OH}{\underset{|}{CH}}{-}\underset{NH_2}{\underset{|}{CH}}{-}\underset{OH}{\underset{|}{CH_2}}}$$

sphingosine (an amino alcohol)



a sphingomyelin

GLYCOLIPIDES (CEREBROSIDES)

Compound lipides which hydrolyze to one fatty acid residue, sphingosine or dihydrosphingosine, and glucose or galactose are called glycolipides. Also called cerebrosides, these compounds occur in relatively large amounts in brain tissue and in smaller amounts in virtually every other organ of the body. Physically they are white solids which are insoluble in ether and water but soluble in hot alcohol and pyridine. In structure, the glycolipides are quite similar to the sphingomyelins except that phosphoryl choline is replaced by galactose or glucose. The fatty acids found in cerebrosides are principally those containing 24 carbon atoms. For example, the cerebroside, kerasin, is a galactolipide containing lignoceric acid while cerebron is a galactolipide containing hydroxylignoceric acid.

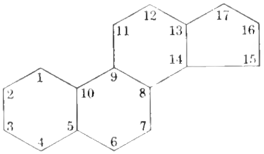
Under certain pathological conditions (for example, Gaucher's disease) large amounts of glucose-containing cerebrosides are deposited in the spleen. Glucolipides are also found in small amounts as normal constituents of almost every organ of the body.

WAXES

These compounds, like the fats, are simple lipides. They differ from glycerides, however, in that they are esters of high-molecular-weight alcohols instead of glycerol. In general, these alcohols are long-chain monohydric alcohols, such as cetyl alcohol ($C_{16}H_{33}OH$), melissyl alcohol ($C_{30}H_{61}OH$), and carnaubyl alcohol ($C_{24}H_{49}OH$). Sometimes the alcohol involved is dihydric as in the case of cocceryl alcohol ($C_{30}H_{60}(OH)_2$). Waxes may be animal or vegetable in origin. While their solubilities are similar to those of fats, the waxes, in general, have appreciably higher melting points. They may be saponified like the fats but with much greater difficulty. The waxes are quite widespread in nature, but as a rule they never occur abundantly. Many natural waxes are mixtures of esters. Beeswax, which is one of the best known of this group, contains melissyl alcohol ($C_{30}H_{61}OH$), combined with palmitic ($C_{16}H_{32}O_2$), cerotic ($C_{26}H_{52}O_2$), and melissic ($C_{30}H_{60}O_2$) acids. Waxes are found in thin layers covering the surfaces of the stems or stalks of many plants, where they function as a protective waterproof coating. The "bloom" on many fruits has been identified as a wax or a wax-like substance.

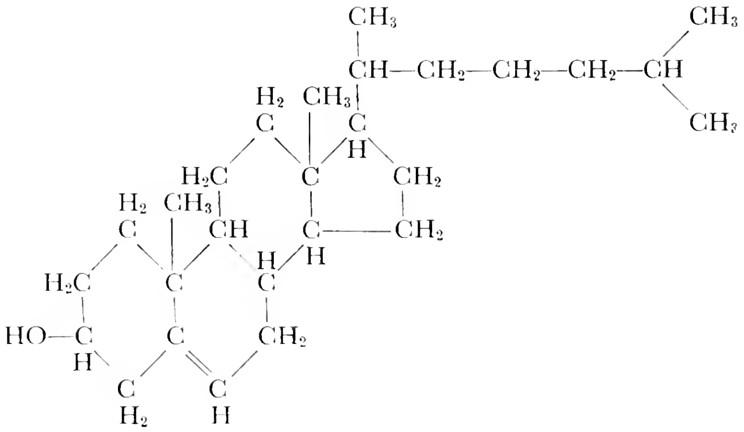
STEROLS

Although the word *sterol* literally means solid alcohol, the term has been limited in recent years to include only certain cyclic alcohols of high molecular weight. Sterols contain a characteristic cyclic nucleus made up of three six-membered rings and one five-membered ring which are arranged and numbered as shown in the following diagram:



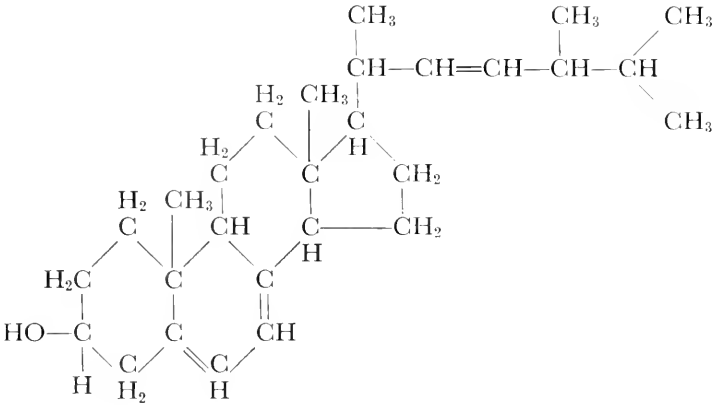
Sterols occur abundantly in plant and animal tissues as well as in yeasts and molds. On the other hand, they have not been shown to occur in bacteria. Although all sterols have the characteristic steroid nucleus shown, they differ markedly from each other with respect to the rest of their structure. Some sterols are saturated, some are unsaturated; some contain one hydroxyl group, whereas others contain several. In addition, sterols differ from each other with regard to the complexity of the side chains attached to the nucleus. Finally, sterols may occur free in nature, or combined with a fatty acid or a carbohydrate.

Cholesterol is probably the best-known member of the sterols. It may be obtained in pure form by extracting human gallstones with hot alcohol containing some potassium alcoholate. The cholesterol crystallizes from the alcoholic solution in rhombic crystals, which, in chloroform solution, are levorotatory. When heated *in vacuo* at about 300°C., cholesterol may be distilled or sublimed without decomposition. It is easily dissolved in fats and in bile. On account of the fact that it is an alcohol and not an ester, cholesterol cannot be saponified; consequently it can be separated from fats following saponification by adding a suitable fat solvent such as ethyl ether. The water-soluble soaps remain in the water solution, while the ether-soluble sterol is found in the supernatant ether layer.



cholesterol

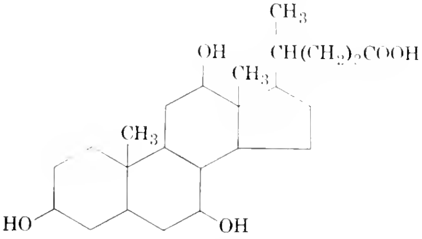
Ergosterol, a sterol which is the precursor of one of the D vitamins (see page 355), was first solated from ergot, a fungus which grows on plants, especially rye. This sterol occurs in a number of food materials in amounts so small that its presence is difficult to detect. When exposed to ultraviolet light, ergosterol gives rise to several new compounds. One of these, calciferol, has been shown to possess antirachitic properties. In chemical structure ergosterol differs from cholesterol as shown by the formula on page 94.



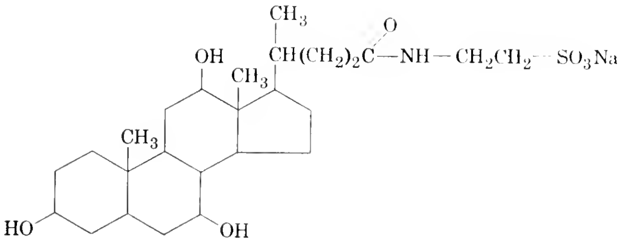
ergosterol

A number of sterols have been isolated from plants. Among these are sitosterol, found in wheat embryo, barley, corn, and cottonseed; stigmasterol, isolated from coconut and soybeans; and spinasterol from spinach and cabbage. All 3 sterols mentioned have 29 carbons in the molecule, each having a 10-carbon side chain. They differ only with respect to the number and position of the double bonds in the molecule.

Bile contains a number of steroids, the most abundant of which is cholic acid (3,7,12-trihydroxycholanic acid). Most frequently the carboxyl group of cholic aid is conjugated with glycine or taurine to



cholic acid



sodium taurocholate
(a bile salt)

form glycocholic or taurocholic acid. The sodium salts of these acids are powerful emulsifying agents which readily form water-soluble complexes with fatty materials, thus aiding in the digestion and absorption of the lipides in the animal.

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The Biochemistry of the Lipids. H. B. Bull. Burgess Publishing Co., Minneapolis, 1935.

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The Chemistry of Lipids of Biochemical Significance. J. A. Lovern. John Wiley & Sons, New York, 1955.

Proteins

5

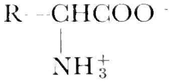
Proteins are essential constituents of all living cells. In fact they appear to be involved in practically all the structures and functions of all cells. Owing to the great molecular size and exceedingly complex structure of proteins, it is deemed advisable to consider first the amino acids. These compounds are combined to form the larger structures, peptides and proteins.

AMINO ACIDS

Amino acids are organic compounds having at least one amino or imino group and one carboxyl group. Other functional groups may be present. The chemical properties of these compounds are those of the amino, carboxyl, and other functional groups with certain special interactions due to the presence of these different structures in a single molecule. Certain of these intramolecular interactions are chiefly responsible for the biological importance of the amino acids.

Occurrence

The almost limitless number of theoretically possible amino acids fortunately does not occur naturally. The natural examples are found both in free and in combined form. Most have the amino group attached to the carbon atom alpha to the carboxyl group. Thus a majority fit the general formula



$$R{-}\underset{\displaystyle NH_3^+}{\underset{|}{C}}HCOO^-$$

where the two functional groups are written in their ionic forms for reasons presented on page 98. R represents any one of a great variety of structures. In the amino acids with imino instead of amino groups, one of the hydrogen atoms of the latter is replaced, usually either by a methyl group or by connection to the R group to form a pyrrolidine ring as in proline. In a few cases other substituents are involved or additional hydrogens are replaced.

Dated lists of the known naturally occurring amino acids show continuous changes. Some compounds have been isolated that are now believed not to occur in living systems but apparently arose during chemical treatments of isolation and identification. On the other hand, the number of known natural amino acids has increased steadily, especially in the last 10 years. New examples will undoubtedly be discovered but probably at a decreasing rate.

A partial list of the present day includes 88 amino acids identified from at least one biological source. A number of these compounds are thought to have limited distribution, occurring in but few organisms as components of alkaloids, antibiotics, or hormones, for example. Many others participate in only the complex metabolic processes of cells. These latter amino acids are widely distributed, of course, usually in free form and in rather small amounts. Still other examples are widely distributed, functioning in special roles like the first group, in metabolism like the second, and in addition as components of proteins. The members of this last group are among the most abundant organic substances, though often in combined form.

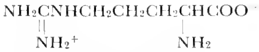
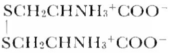
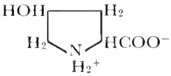
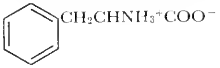
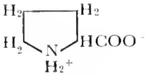
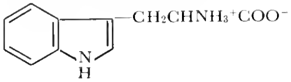
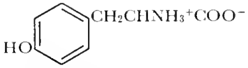
The names and structures of the amino acids combined to form most proteins are listed in Table 5–1. It must be emphasized that many other amino acids have been observed in other materials and studied, but space does not permit their tabulation or discussion here. Hydroxyproline is included as is customary, although this amino acid has been demonstrated only in the proteins of the widespread gelatin-collagen group, where it is quite abundant.

Electrochemical Properties

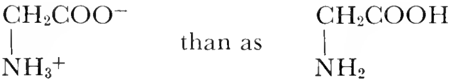
The electrical charges on amino acids influence the properties markedly. These charges arise from the fact that amino acids possess both basic and acidic groups and are thus amphoteric. In addition, other ionizable groups are present in certain cases and all play important roles.

All molecules with both acidic and basic groups might exist as uncharged molecules, as dipolar ions with ionic charges of opposite sign, or as a mixture of these. Many different lines of evidence indicate

TABLE 5–1. The Amino Acids Commonly Occurring in Proteins

Amino Acid	One Scientific Name (Acids)	Structure
Alanine	α-Aminopropionic	$CH_3CHNH_3^+COO^-$
Arginine	α-Amino-δ-guanidino-*n*-valeric	 $\underset{NH_2^+}{\underset{\parallel}{NH_2C}}NHCH_2CH_2CH_2\underset{NH_2}{\underset{\vert}{CH}}COO^-$
Asparagine	Aminosuccinic (amide)	$NH_2OCCH_2CHNH_3^+COO^-$
Aspartic acid	Aminosuccinic	$HOOCCH_2CHNH_3^+COO^-$
Cysteine	α-Amino-β-thiolpropionic	$HSCH_2CHNH_3^+COO^-$
Cystine	Di(α-aminopropionic)-β-disulfide	 $\begin{array}{l}SCH_2CHNH_3^+COO^-\\ \vert\\ SCH_2CHNH_3^+COO^-\end{array}$
Glutamic acid	α-Aminoglutaric	$HOOCCH_2CH_2CHNH_3^+COO^-$
Glutamine	α-Aminoglutaric (amide)	$H_2NOCCH_2CH_2CHNH_3^+COO^-$
Glycine	Aminoacetic	$CH_2NH_3^+COO^-$
Histidine	α-Amino-β-imidazolopropionic	
Hydroxyproline	γ-Hydroxypyrrolidine-α-carboxylic	
Isoleucine	α-Amino-β-methyl-*n*-valeric	 $CH_3CH_2\underset{CH_3}{\underset{\vert}{C}}HCHNH_3^+COO^-$
Leucine	α-Aminoisocaproic	$(CH_3)_2CHCH_2CHNH_3^+COO^-$
Lysine	α,ε-Diamino-*n*-caproic	$H_2NCH_2CH_2CH_2CH_2CHNH_3^+COO^-$
Methionine	α-Amino-γ-methylthiol-*n*-butyric	$CH_3SCH_2CH_2CHNH_3^+COO^-$
Phenylalanine	α-Amino-β-phenylpropionic	
Proline	Pyrrolidine-α-carboxylic	
Serine	α-Amino-β-hydroxypropionic	$HOCH_2CHNH_3^+COO^-$
Threonine	α-Amino-β-hydroxybutyric	$CH_3CHOHCHNH_3^+COO^-$
Tryptophan	α-Amino-β-indolepropionic	
Tyrosine	α-Amino-β-(*p*-hydroxyphenyl)-propionic	
Valine	α-Aminoisovaleric	$(CH_3)_2CHCHNH_3^+COO^-$

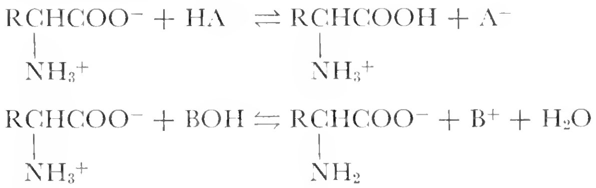
beyond doubt that ordinary amino acids exist in both solid state and simple aqueous solution as dipolar ions or zwitterions. Calculations show that an aqueous solution of glycine, for instance, can have no more than one part of uncharged molecules in a hundred thousand parts of dipolar ions. Hence, it is more accurate to write the structure of glycine as



$$\begin{array}{l}CH_2COO^-\\ \vert\\ NH_3^+\end{array}\quad \text{than as}\quad \begin{array}{l}CH_2COOH\\ \vert\\ NH_2\end{array}$$

In typical amino acids, the proton has migrated from the carboxyl to the amino group, the actual structures thus becoming carboxylate and ammonium ions. This method of writing the structure is preferred and is adopted here to assure constant recognition of the presence of the charged groups and their special contributions to the properties of these systems.

As typical amphoteric compounds, amino acids react with both acids and bases as follows:



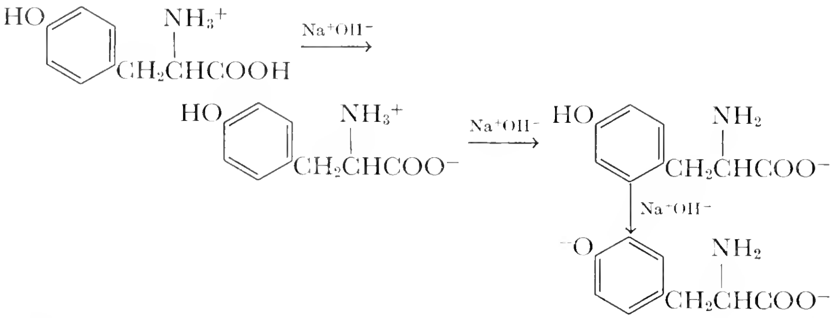
$$\underset{\substack{|\\ \mathrm{NH_3^+}}}{\mathrm{RCHCOO^-}} + \mathrm{HA} \rightleftharpoons \underset{\substack{|\\ \mathrm{NH_3^+}}}{\mathrm{RCHCOOH}} + \mathrm{A^-}$$

$$\underset{\substack{|\\ \mathrm{NH_3^+}}}{\mathrm{RCHCOO^-}} + \mathrm{BOH} \rightleftharpoons \underset{\substack{|\\ \mathrm{NH_2}}}{\mathrm{RCHCOO^-}} + \mathrm{B^+} + \mathrm{H_2O}$$

where HA and BOH represent acid and base, and A^- and B^+ anion and cation, respectively. Thus in acid solution the carboxylate ion acquires a proton, and the amino acid becomes an ammonium salt of the acid. Conversely, in alkaline solution the ammonium ion loses a proton, and the amino acid becomes the anion of a salt. These reactions are quite reversible when the pH of the solution is shifted suitably. Therefore, amino acids serve as buffers in certain ranges of pH and tend to prevent pH change when acid or base is added. This class of compounds contributes to the buffer capacity of many biological fluids and is used in certain artificial buffers, particularly in regions of either high or low pH.

Whenever a substance exists as a zwitterion with charges of equal magnitude but opposite sign, it is said to be isoelectric. The pH at which this state occurs is called the isoelectric point. Actually the ionization constants of most of the polar groups involved are far enough apart to allow isoelectric molecules over a range of pH, which is nevertheless still referred to rather inaccurately at the isoelectric "point." All dipolar ions large and small have characteristic isoelectric points which depend upon the nature and number of the ionizable groups present and to some extent upon the solvent and additional solutes in the system.

Other ionizable groups merely introduce another step into the reaction process. Consider tyrosine as a typical example, starting with it in strongly acid solution, where it exists as the ammonium ion, shown on page 100.



At 25°C. the first step is half complete at pH 2.20, still quite acid. The second step is half complete at pH 9.11, and the first reaction was complete much below this moderately alkaline value. Step three is half complete at pH 10.07. In very alkaline solutions tyrosine thus exists as a doubly charged anion.

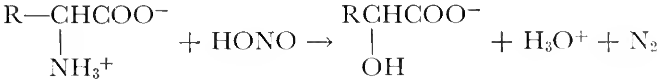
These additional ionizable groups include the phenolic hydroxyl of tyrosine, secondary carboxyl groups as in aspartic and glutamic acids, extra amino as in lysine, and the basic guanido and imidazolium groups of arginine and histidine. (See Table 5–1 of amino acids.) These structures are extra functional groups and are extremely important in the biochemistry of these compounds.

When an aqueous solution of an amino acid like glycine is placed in an electric field, the glycine does not move, since it has charges of opposite sign and equal magnitude at its isoelectric point. In this way glycine differs from simple ions which migrate rapidly toward the electrode of opposite sign. This phenomenon of migration in an electric field is called electrophoresis, or ionophoresis when the electrolytes are small. Glycine will, of course, migrate in the field when the pH is changed enough to remove either one of the ionic charges. Other amino acids, like aspartic acid or lysine, already have an unequal number of opposite charges. In pure water these compounds move in the direction dictated by the preponderant charge type. Taking advantage of the fact that different amino acids have different numbers and types of ionizable groups, mixtures of amino acids may be separated into the individual compounds or into simpler mixtures by means of ionophoresis. Special methods incorporating other effects permit the complete separation of the amino acids in mixtures.

Chemical Properties

Only a very few of the known chemical properties of amino acids can be mentioned here. One of the typical reactions of acids is the formation of amides. Since amino acids contain both necessary functional groups, two molecules should be able to combine with each other. This process occurs, of course, and leads to the formation of peptides and proteins. This biologically important chemical property of amino acids is discussed more extensively under the peptide bond (page 104).

The ammonium ions may be replaced by hydroxyl groups by means of various reactions, the most common employing nitrous acid:



$$\underset{\substack{|\\ NH_3^+}}{R{-}CHCOO^-} + HONO \rightarrow \underset{\substack{|\\ OH}}{RCHCOO^-} + H_3O^+ + N_2$$

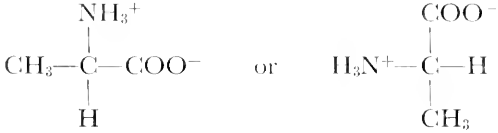
At a suitable pH this reaction is quantitative, and measurements of the nitrogen gas indicate the number of amino groups or monosubstituted ammonium ions present. In fact, this determination can be applied to peptides and proteins. Biochemical systems carry out the same sort of replacement reaction in the metabolism of amino acids. In cells the actual mechanism is different, but hydroxy acids and keto acids are produced in large amounts. Moreover, the process is reversed by cells using ammonia and keto acids to form amino acids (see page 464).

Other chemical reactions occur and many are carried out in biochemical operations of various kinds discussed in the sections on metabolism (pages 463–494). Finally, as ionic compounds, amino acids tend to be soluble in polar solvents like water and insoluble in non-polar solvents. Such solubility behavior is required by the universal biological roles of water.

Optical Isomerism

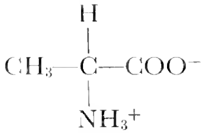
Most but not all of the naturally occurring amino acids possess asymmetric carbon atoms. Hence there are possibilities for optical isomerism in this group of compounds. In fact, those examples with asymmetric carbons occur in given sources as particular isomers. It is believed, therefore, that optical specificity of amino acids is preserved with regard to each specific occurrence. In other words, a particular peptide with D-alanine in it contains the D form only. The alanine of proteins, on the other hand, is always of the L form.

The conventional representation of the configuration of carbohydrates was explained on page 59. For amino acids the common two-dimensional scheme is illustrated with alanine.



$$\begin{array}{ccc} & NH_3^+ & \\ & | & \\ CH_3— & C & —COO^- \\ & | & \\ & H & \end{array} \quad \text{or} \quad \begin{array}{ccc} & COO^- & \\ & | & \\ H_3N^+— & C & —H \\ & | & \\ & CH_3 & \end{array}$$

L-alanine



$$\begin{array}{ccc} & H & \\ & | & \\ CH_3— & C & —COO^- \\ & | & \\ & NH_3^+ & \end{array}$$

D-alanine

This representation places the ammonium ion clockwise from the carboxyl ion in the D forms and counterclockwise in the L forms regardless of the actual optical rotations. Initially this choice was made arbitrarily, but it is now known from X-ray studies that the convention represents the actual projection of the groups in two dimensions.

Several amino acids possess two asymmetric carbon atoms as in hydroxyproline, and more than two isomers are possible. Although the actual configuration of the extra asymmetric atom is not known in most of these cases, there seems to be a pronounced preference for a particular configuration in any given natural source. There are other amino acids with two asymmetric structures but with a point of overall molecular symmetry, as in cystine. Optically inactive *meso* isomers are then possible, but only one such form is known to occur naturally.

Returning to the configuration of the α-carbon atom, we may say that very extensive studies have failed to discover D forms of any of the amino acids in proteins. This apparently complete preference for L isomers in proteins extends to all the great number of individual proteins and species studied. The origin, preservation, and value of this high degree of specificity raise a number of interesting problems, none of them settled and some not even subject to experimentation at present. If, for example, only L-amino acids are available in the diet of an animal, it ought to be easier to synthesize protein from these directly without inverting the configuration. Moreover, the metabolic machinery of the cell should be simpler if a single isomer of each amino acid is handled. In addition, it is felt that specific configurations may be critical in the enzymatic and other biological functions of proteins. These points and other related reasoning suggest the value of optical activity, but real experimental proof is not available.

D-Amino acids do occur in certain peptides and apparently also in free form in very small amounts. Hence animals might consume limited quantities of these isomers. Although D isomers are apparently not absorbed and utilized as well as the much more common L forms, many species can metabolize them to some extent. The process is thought to involve conversion either to optically inactive compounds like keto acids or to L-amino acids at an early stage rather than involving metabolism along a complete pathway parallel to that for L isomers. It is interesting to note that certain compounds containing D-amino acids are toxic. Such substances, often peptides, apparently cannot be split into the individual components and metabolized but as intact units block normal cell reactions and impair cell function. Such compounds are toxic to microorganisms as well as to higher forms, and some have been used as antibiotics when the toxicity for bacteria exceeds that for animals.

Metabolism

Owing to the variety and complexity of the naturally occurring amino acids, the transformations they undergo in the cells of different species are both numerous and diverse. Advances in the knowledge of these reactions are being made very rapidly, and the present information is so extensive that it cannot even be outlined here. However, it is desirable to illustrate some of the known reactions in the metabolism of one set of amino acids. The system chosen (see Figure 20–1) includes six of the amino acids listed earlier as commonly occurring in proteins and four others that function as metabolic intermediates.

When the arrows signifying reaction are double pointed, the particular step is known to be reversible. Single points signify lack of known reversibility. Each reaction stage shown requires at least one enzyme as a catalyst. Some reactions actually are known to be complex, involving additional factors like metals, special phosphate derivatives, coenzymes, and even more than one enzyme. Nearly every compound shown participates in other metabolic sequences not given here, either leading to or coming from other products. Moreover, not all species utilize these same pathways for these compounds, but this scheme does appear to be rather widespread.

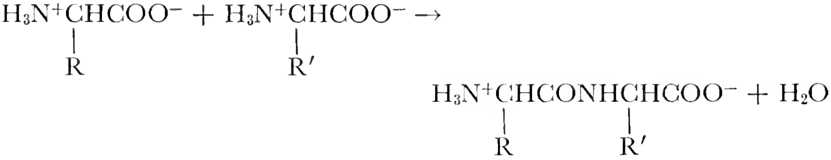
The circular sequence of compounds appearing at the top of this metabolic scheme is called the Krebs, citric acid, or tricarboxylic acid cycle. This part of the system is examined in more detail on page 171. The lower cycle of four amino acids is sometimes referred to as the ornithine cycle. It apparently is the principal source of the urea excreted by most mammals in eliminating waste nitrogen.

Since many cellular processes are either reversible or cyclic, they may serve either in the synthesis or the degradation of the compounds concerned. The mechanisms controlling the directions of such pathways are largely unknown but are thought to depend on the available supply of and demand for the intermediates in the cell. Details of this and other pathways for the metabolism of amino acids are available in any one of a number of reviews devoted to this subject.

PEPTIDES

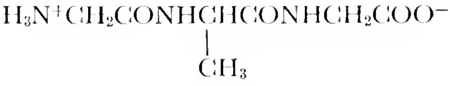
The Peptide Bond

Compounds of this class are made up primarily of amino acids. A few natural substances placed in this group are not composed exclusively of amino acids, but the additional components are limited in both number and size. Hence the fundamental bonds connecting the subunits of the peptides are those joining the amino acids. Such bonds link the carboxylate ion of one amino acid with the ammonium ion of another and are called the peptide bonds. The product is a special type of amide known as a peptide and is represented in the general reaction for the formation of a peptide bond:



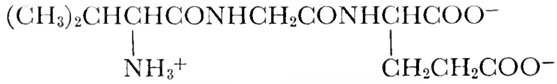
$$H_3N^+\underset{\substack{|\\ R}}{C}HCOO^- + H_3N^+\underset{\substack{|\\ R'}}{C}HCOO^- \rightarrow H_3N^+\underset{\substack{|\\ R}}{C}HCONH\underset{\substack{|\\ R'}}{C}HCOO^- + H_2O$$

In this example the peptide consists of two amino acid subunits or residues and thus is a dipeptide. Tripeptides contain three amino acid residues, and so on to polypeptides with a large number of such residues. Specific names are applied when the nature and relative positions of the amino acid residues are known. These names begin with the amino acid bearing the free α-ammonium ion at one end of the molecule and proceed stepwise to the amino acid carrying the terminal carboxylate ion. In this way a name is based on that of the right-hand amino acid when written as above and prefixed with the names of the substituents in order. When the optical configurations of the individual amino acids are known, they are designated by the appropriate letters. Usually, in writing the structures of peptides, no effort is made to represent the actual configurations of asymmetric carbon atoms because of difficulties in projecting into two dimensions when several asymmetric atoms are involved. The following examples illustrate these principles of nomenclature:



$$H_3N^+CH_2CONH\underset{\substack{|\\ CH_3}}{C}HCONHCH_2COO^-$$

glycylalanylglycine



$$(CH_3)_2CH\underset{\substack{|\\ NH_3^+}}{C}HCONHCH_2CONH\underset{\substack{|\\ CH_2CH_2COO^-}}{C}HCOO^-$$

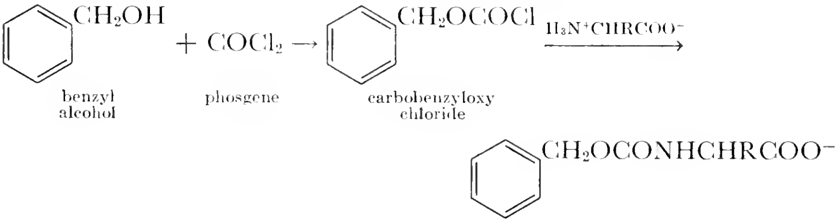
L-valylglycyl-L-glutamic acid

The synthesis of peptides by cells is the subject of much current study, but the mechanisms have not yet been established. However, laboratory syntheses have been developed and many peptides are now available. The method employed in any given case depends primarily on three factors. First, one of the reacting structures, either the carboxylate or ammonium ion, must be activated by modifying it in some way. Second, reaction of the activated molecule with another of its own kind must be prevented. Finally, any additional functional groups of the amino acids involved must be preserved. The last condition is usually met by protecting these additional structures with groups that can be removed later or by choosing a method of activation that does not lead to reaction of the auxiliary structures.

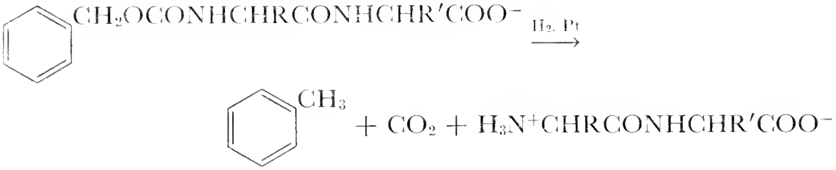
Reaction of activated amino acid molecules with themselves instead of with the second kind of amino acid is always a problem. The difficulty arises, of course, because both structures participating in peptide-bond formation occur in each component molecule. The problem is met in one of two ways. The original reactants may not both be amino acids but may be derivatives forming amides later converted to peptides, as with

$$\mathrm{ClCHRCOCl + H_3N^+CHR'COO^- \rightarrow}$$
$$\mathrm{ClCHRCONHCHR'COO^- \xrightarrow{NH_4OH} H_3N^+CHRCONHCHR'COO^-}$$

where R and R′ stand for any of the side groups typical of amino acids. Often the necessary chloro acids are not available. Then it is usual to protect either the ammonium or carboxylate structure with a group that can be removed later. In the latter case, simple esters are usually made. A number of protecting groups are used to cover the ammonium ion, most of them forming complex amides as in



After this derivative has been coupled to a second amino acid, the protecting group may be removed by reduction, giving the peptide in high yield.



When the appropriate groups have been protected, one of several available methods of activation is applied to bring about the formation of the peptide bond. Three procedures are cited as examples, with R″ and R‴ representing protecting groups that are removed later.

$$\mathrm{R''NHCHRCOOH} \xrightarrow{\mathrm{SOCl_2}} \mathrm{R''NHCHRCOCl} \xrightarrow{\mathrm{H_2NCR'COOR'''}} \mathrm{R''NHCHRCONHCHR'COOR'''}$$

$$\begin{aligned} &\mathrm{R''NHCHRCOOEt} \xrightarrow{\mathrm{H_2NNH_2}} \mathrm{R''NHCHRCOHNNH_2} \xrightarrow{\mathrm{HNO_2}} \mathrm{R''NHCHRCON_3} \\ &\qquad \big\downarrow \mathrm{H_2NCHR'COOR'''} \\ &\mathrm{R''NHCHRCONHCHR'COOR'''} + \mathrm{HN_3} \end{aligned}$$

$$\begin{aligned} &\mathrm{NH_2CHRCOOR'''} + \underset{\text{tetraethyl-pyrophosphite}}{\mathrm{(EtO)_2POP(OEt)_2}} \xrightarrow[\text{diethyl-phosphite}]{\mathrm{(EtO)_2POH}} \\ &\mathrm{(EtO)_2PNHCHRCOOR'''} + \mathrm{(EtO)_2POH} \\ &\qquad \big\downarrow \mathrm{R''NHCHRCOOH} \\ &\mathrm{R''NHCHRCONHCHR'COOR'''} + \mathrm{(EtO)_2POH} \end{aligned}$$

Of these, the first two procedures represent activation of the carboxyl group and the last method represents activation of the amino group. The last reaction can be used to activate the carboxyl group instead of the amino group if the tetraethylpyrophosphite is added to the N-acylamino acid derivative first. Many other methods have been used. Some have general utility; some can be applied only to the synthesis of particular peptides. A wide variety of protecting and activating methods is necessary in view of the diversity of the amino acids involved and the differences in their chemical properties.

Occurrence

It is theoretically possible to combine the rather sizable number of naturally occurring amino acids into an enormous variety of peptides differing in size, composition, or the sequence of the component amino acids. It is probable that the number of peptides occurring naturally in free form is large. However, methods of isolation, separation, and structure study have not been available long enough to allow even a rough estimate of the number. The presence of free peptides has been demonstrated in biological fluids like blood, urine, and plant juices, but work has not progressed far enough to permit enumeration and identification of the compounds involved.

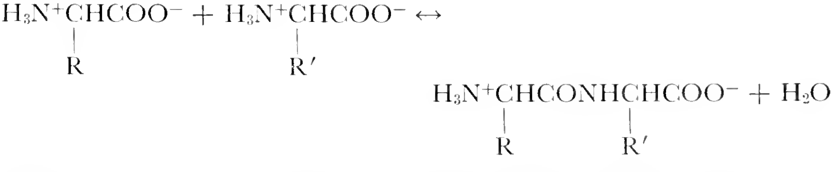
As intermediates in the hydrolysis of proteins, peptides are formed in great abundance and variety and have been studied extensively. The digestion of proteins by animals and microorganisms alike depends upon catalytic hydrolyses with proteolytic enzymes. These catalysts specifically break certain peptide bonds, forming peptides whose size and structure depend upon the nature of the protein split and the enzyme participating. Hydrolysis of proteins *in vitro,* meaning outside a living system, has been studied widely in work on protein structure. Many peptides have been isolated and their structures established, revealing the possibility of occurrence of an almost infinite variety.

Peptides are at least potential intermediates in biological protein syntheses. If so, then they might occur in still other structural types than those demonstrated during protein breakdown. In addition, a number of peptides have been isolated from special sources and possessing special physiological actions. The sources of some members of this group are listed below in connection with their structures and actions.

Structures and Properties

Peptides consist primarily of amino acids linked together through peptide bonds. Other structures of various kinds may also be involved in certain special peptides and are usually present as amides. These groups may be represented by ethanolamine, one of several different simple organic acids, glucuronic acid, or a ribose derivative. The contribution of these additional components to structure and properties can be inferred from their nature and is quite specific; therefore it will not be considered.

The specialized chemistry of peptides is the chemistry of the peptide bond. For each such bond formed at physiological values of pH,



$$H_3N^+\underset{\substack{| \\ R}}{C}HCOO^- + H_3N^+\underset{\substack{| \\ R'}}{C}HCOO^- \leftrightarrow H_3N^+\underset{\substack{| \\ R}}{C}HCONH\underset{\substack{| \\ R'}}{C}HCOO^- + H_2O$$

two charged groups disappear. Conversely, two charges appear when a peptide bond is hydrolyzed. The greater the number of ionic charges present in solution, the higher the dielectric constant of the solution. Both solubilities of materials and the rates of many reactions are influenced by dielectric constant. Hence, the number of charges and therefore the size of peptides indirectly influence many metabolic processes.

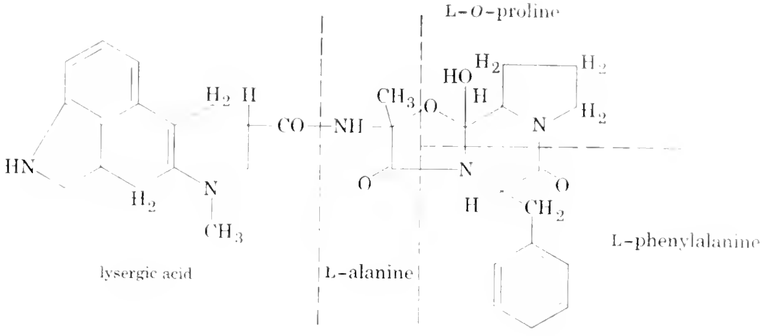
Most ordinary amides are not very polar and not very soluble in water. The charged groups of peptides contribute polarity, and water solubility becomes commonplace. However, in peptides the ionic charges are further apart than in the amino acids themselves. Thus one would expect somewhat less effect of the charges on each other than when close together. One result of the separation of charges is the alteration of the ionization constants of the carboxyl and amino groups. For example, the constants for glycine are $K_1 = 10^{-2.3}$ and $K_2 = 10^{-9.8}$, whereas those for glycylglycine are $K_1 = 10^{-3.1}$ and $K_2 = 10^{-8.1}$. These differences lead to differences in the buffering action of the two kinds of compounds, one kind being more effective in the pH ranges of about 1.5 to 3.1 and 9.0 to 10.6, while the other covers the pH ranges of 2.3 to 3.9 and 7.3 to 8.9.

As molecules become larger, they diffuse more slowly. This effect is increased, of course, when a semipermeable membrane serves as a barrier to diffusion. Hence large peptides diffuse out of cells more slowly than either smaller peptides or amino acids. Perhaps these differences determine in part the concentrations inside cells and influence the forms in which waste nitrogenous materials are excreted by marine forms and higher animals.

Biological Action

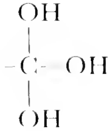
Peptides play a number of biological roles. They may possibly serve as intermediates in the formation of proteins and in this way would play fundamental roles in almost all biological activities. However, some current investigators believe that proteins are formed directly from amino acids without intervening peptides. Others do not agree with this concept; clarification of the problem must await further experimentation.

Certain plant peptides are placed in the diverse group of compounds called alkaloids. The peptide members of this class produce striking effects when administered in small amounts to animals. Most of these peptides with pronounced pharmacological properties have been isolated from fungi, although they may turn up in numbers in higher plants, since one example has been obtained from mistletoe. Ergotamine, shown in the accompanying structural formula, is a pep-



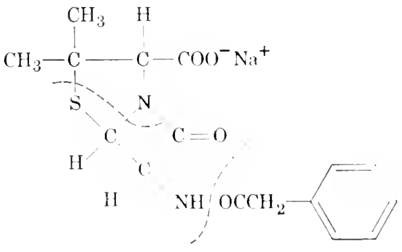
ergotamine

tide alkaloid from rye ergot. Ergot is a fungus infesting rye and producing toxic symptoms in animals that ingest it. Of the four components in this structure, L-*o*-proline is unknown in free form, being a member of the group of ortho acids known from their derivatives and represented with carboxyl groups in the completely hydrated form



$$-\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}-OH$$

Members of another large group of peptides possess antibacterial activities and occur in many materials, most of the known cases being from fungi and bacteria. Many are powerful antibiotics but most are too toxic for medical use. However, the group does include the penicillins. Penicillin G is the most common and may be written thus:



where the dotted lines are used to reveal the components. The top portion has the structure and configuration of D-valine, the middle part is D-cysteine, and the lower component is phenylacetic acid. The other penicillins possess analogous structures but with different acids in amide linkage with the cysteine. Two of the rings are formed by the peptide bond between cysteine and valine plus two additional linkages not usually encountered in peptides.

Another type of peptide which has been isolated from the fungus *Fusarium lycopersici* produces the symptoms of a tomato blight and kills this plant. A number of metabolic disturbances occur, although it is difficult to decide which are primary and which are secondary. Though not yet conclusively proved, the structure is thought to involve asparagine and an addition product of glycine and pyruvic acid.

$$\mathrm{H_2NOCCH_2\underset{\displaystyle COOH}{C}HNH}\;\vdots\;\mathrm{OCCH_2NH}\;\vdots\;-\overset{\displaystyle CH_3}{\underset{\displaystyle COOH}{\mathrm{C}}}-\mathrm{OH}$$

lycomarasmin

By contrast, other peptides serve as growth factors and either are required for growth or increase it. One of these, the citrovorum factor and its relatives, is discussed as folic acid on page 344. Another group stimulates the growth of a variety of microorganisms. These peptides are called strepogenins and have been isolated after the partial hydrolysis of any one of several proteins. One strepogenin is the pentapeptide serylhistidylleucylvalylglutamic acid, and another is a heptapeptide having the same sequence attached to alanylleucine. The mechanism by which growth is stimulated is still unknown, but replacement by a simple mixture of the corresponding amino acids does not suffice. In a similar way certain peptides may be desirable, if not essential, in the diets of higher animals, but conclusive experiments have not been performed.

The higher animals do synthesize certain peptides essential to their welfare which serve as hormones. The possible medical importance of these compounds has stimulated interest in them and led to a great deal of study, including successful structural and synthetic work. These peptides are discussed along with other hormones on pages 311 to 325.

A final example is the peptide glutathione, believed to occur in all cells. It is a tripeptide with the structure

$$^{-}\mathrm{OO\underset{\displaystyle NH_3^+}{C}CHCH_2CH_2CONH\underset{\displaystyle CH_2SH}{C}HCONHCH_2COO^-}$$

γ-L-glutamyl-L-cysteinylglycine

Note the unusual peptide bond involving the γ-carboxyl group of glutamic acid instead of the much more common α-peptide bond. Glutathione is believed to be important in controlling the oxidation-reduction potential of the cell, helping to keep a variety of critical —SH groups in the reduced form. This peptide may be a key intermediate in electron-transfer systems. It also is a required cofactor in certain enzyme reactions, and it can serve in peptide syntheses as a source of glutamic acid, which occurs in the peptide with both α-carboxyl and amino groups free.

Effective methods for the isolation and study of peptides have been available for only about 10 years. Hence there must be a great deal more to be learned of the biochemistry of these compounds, and new roles will undoubtedly be discovered.

PROTEINS

Proteins are combinations of amino acids and are still larger than natural peptides. They often contain some other structure in addition to the amino acids, these latter being united principally by means of peptide bonds. Proteins always appear to be giant or macromolecules with molecular weights in the range of 10^4 to 10^7. Although this class is difficult to define, one may say that a protein is a macromolecule of biological origin consisting largely of amino acids condensed via peptide bonds.

Composition and Structure

Although there are many naturally occurring amino acids, only the 22 listed in Table 5–1 have been isolated from proteins. Moreover, only the L isomers have been definitely found. Reports of D isomers seem to refer to artifacts of isolation arising from racemization of one of the natural forms. In addition, it is now quite clear that not all proteins contain all of even these 22 amino acids. As mentioned earlier, hydroxyproline is found only in gelatins and collagens. There are many proteins, especially smaller ones, which do not contain all the amino acids listed. For example, the protein hormone insulin is without hydroxyproline, tryptophan, cysteine, and methionine. However, these particular amino acids do occur in other proteins.

Even though the list of amino acids in proteins is limited, the possible variety of protein structures is not. The number of each kind of amino acid per protein molecule is widely variable. The sequence of the amino acids in the peptide chain is specific for each protein and potentially capable of enormous diversity. Finally, proteins may differ in the number of peptide chains per protein molecule. A peptide chain is a sequence of amino acids condensed by way of peptide bonds.

Two or more such chains may be held together with —S—S— bonds of cystine, with each half of the cystine being in a different peptide chain. Salt formation between ionic groups of different chains and hydrogen bonds involving $>\mathrm{NH}$ and $\mathrm{O{=}C}<$ probably also help hold peptide chains together in the protein molecule.

Under physiological conditions, insulin appears to have four separate peptide chains, two each of two kinds. This particular protein dissociates into equal halves when the environment is altered. More drastic treatment breaks apart the individual peptide chains. Although the available information of this type is rapidly increasing, little is known about the numbers of peptide chains in most proteins.

Even less is known about the sequences of the amino acids in proteins. Only in the insulins of five species have the arrangements been completely worked out. Insulins from cattle, swine, sheep, horses, and whales have been studied. All are alike, with respect to one chain. All differ in the other type of peptide chain with the differences occurring at three adjacent sites along the sequence. The amino acids interchanged among these positions are alanine, serine, valine, threonine, isoleucine, and glycine. Insulin is the first protein for which the species differences have been chemically located with exactness.

The problem of the amino acid composition of proteins has been studied for many decades. Yet even the best available results are never in complete agreement. Basically the problem is one of determining the number of amino acids of each kind present in the protein molecule. For proteins exceeding 10^5 in molecular weight, some of the amino acids are so abundant that modern assay methods cannot yield the number of these correct to the nearest whole number. With the less common amino acids, the problem of accuracy is not so severe and may not be a factor. However, most determinations require that the amino acid be in free form. Hence the proteins must be hydrolyzed, but unfortunately some amino acids are not completely stable during hydrolysis. Even worse, certain amino acids are less stable in the form of peptides than they are in the free state, and controlled degradation studies become inaccurate. Thus losses are uncertain, and final estimates of composition reflect variations in methodology. Insulin was perhaps the most intensively analyzed of all proteins, but each laboratory reported somewhat different compositions. None agreed exactly with the results obtained from the presumably correct studies of the amino acid sequences in the peptide chains.

Many kinds of protein molecules are believed to contain nothing but amino acids in combined form with perhaps water of hydration and some associated cations and anions. However, other proteins possess additional structures attached to the peptide chains. These extra functional groups include certain metal cations, carbohydrates, lipides, phosphate as an ester, one of a group of complex organic compounds called porphyrins (see page 470), and the nucleic acids discussed on pages 120 *et seq.*

Classifications and Nomenclature

The study of proteins is an old one, and description of the work required that names be devised. As is customary in a new field, the earliest names were hit or miss, but the use of the ending *in* was generally employed for proteins in combination with randomly chosen roots, sometimes reflecting the source of the protein or some particular property of it. This system is still applied except that the suffix *ase* is now used in naming the members of the very large catalytically active group of proteins called enzymes. The names of some enzymes known long ago, like pepsin and trypsin, still bear the ending more characteristic of non-enzymatic proteins.

As the number of known proteins increased, it was natural to compare and contrast their properties, thus leading directly to classification of proteins. Classifications are always based on properties, and different systems developed emphasizing different groups of properties. As more was learned of proteins, their great diversity became more apparent; the class covers a great range of properties of many kinds. Hence proteins alike in one respect differ in others and could be classified together or into separate categories, depending upon the focus of attention. Thus many classifications have been developed, none satisfactory for all purposes or even very many purposes. Some of the bases used for systems of classification grouped proteins in various ways according to their sources, solubilities, compositions, structures, or biological functions. A scheme based on any one of these failed to impart the information conveyed by the others. It now seems impossible to conceive of a classification sufficiently flexible to serve all common needs. Moreover, the study of proteins has progressed so far that the field as a whole is mastered by no individual. Hence, each worker tends to concern himself with a limited number of proteins related in some way without worrying much about the classification of the remaining vast array of different proteins.

Nevertheless, a sort of hybrid classification should be set forth, not so much because this is the best system, but because it contains much of the terminology of protein chemistry.

I. Simple proteins. Contain only the ordinary α-amino acids without other organic or inorganic structures. This major group is then further subdivided on the basis of solubility.

A. Albumins. Soluble in water and dilute salts, acids, and alkalis. Coagulated by heat, although there are great variations in sensitivity. Egg albumin, serum albumin from blood, and many enzymes are examples.

B. Globulins. Most are insoluble in water but dissolve in salt solutions of moderate concentration. Less soluble in concentrated salt solutions than albumins. Heat coagulated. Obviously the globulins may grade into the albumins, and the classification is not clear cut. Many seed proteins, myosin from muscle, and the antibodies of blood are typical examples.

C. Glutelins. Insoluble in water and salt solutions at isoelectric pH values. Dissolve in dilute acids and alkalis. A number of seed proteins, particularly those of cereal grains, are typical examples.

D. Prolamines. Insoluble in water but dissolve in 70 to 80 per cent ethanol. Have relatively few polar groups, are usually high in proline and amide groups, and are also common in seeds.

E. Scleroproteins or albuminoids. Insoluble in all solvents except when coincident with decomposition. Include structural and fibrous proteins.

F. Histones. Soluble in water but insoluble in dilute ammonia. Biologically associated with certain acidic structures and are themselves quite basic, being high in lysine and arginine. Examples include globin of hemoglobin and the protein components of most nucleoproteins.

G. Protamines. Small basic proteins not coagulated by heating and soluble in most aqueous systems. Known only in combination with nucleic acid in the sperm of fish.

II. Conjugated proteins. Yield other materials than amino acids on hydrolysis. Often the special group may be detached without destruction of the protein moiety, which may then be classified according to Part I above. Many conjugated proteins have quite specific functions.

A. Phosphoproteins. Contain phosphate groups linked through ester bonds to hydroxyamino acids. Casein and pepsin are typical.

B. Glycoproteins or mucoids. Conjugated with carbohydrate, usually macromolecular polysaccharides containing acetylglucosamine, other sugars or sugar acids, and sulfate or phosphate esters. Tend to have unusual properties based on the presence of many polar groups. Not much is known about them, but they occur in eggs and blood. The gonadotropic hormone is an example.

C. Chromoproteins. Possess structures absorbing visible light and are colored. Color may be due to metals like copper and vanadium or metals with organic groups as in the iron and magnesium porphyrins. Include many respiratory pigments and a large number of enzymes.

D. Lipoproteins. Conjugates of lipides and proteins, the lipide not extractable with ether. They are widely distributed, especially in animal tissues like eggs, brain, and blood. The lipides may be both large and complex aggregates.

E. Nucleoproteins. Conjugates of nucleic acids and proteins. Considered in a separate section on page 120.

Another group of related materials consists of derived proteins formed by modifying the native or natural proteins. Coagulated proteins are the insoluble products obtained by heat or alcohol treatment. Metaproteins are the slightly modified products obtained on treating native proteins with dilute acid or alkali at temperatures below about 60°C. Proteoses are formed when degradation by acid, alkali, or enzymes proceeds beyond the metaprotein stage. Protesoses are not coagulated by heat, tend to be soluble in most aqueous systems but not in saturated ammonium sulfate, and are often toxic when injected into animals. Peptones are formed by still more extensive hydrolysis, cannot be salted out, and are of relatively small size. They correspond to peptides of moderate size.

Other systems of classification employ terms with fairly obvious meanings to indicate source, function, shape, and the like. As examples, one may list, respectively, blood proteins, respiratory proteins, and globular proteins. No particular discussion of such terminology appears necessary.

Chemical and Physical Properties

The known chemical properties of proteins are fundamentally those of the functional groups present. Thus the properties depend upon those of the amino acid components with some modification in reactivity and steric factors resulting from the presence of a peptide chain. As expected, the free amino groups of proteins may be acylated, replaced with hydroxyl groups, combined with formaldehyde, converted to substituted ammonium ions by treatment with acids, or undergo many of the other standard reactions of amines. Carboxyl groups can be used as proton donors, esterified, incorporated into mixed anhydrides, reduced, or converted into amides, peptides, or acyl halides.

Hydroxyl groups of serine and threonine are typical of the alcohols and react in the same ways. The sulfhydryl group of cysteine and the sulfide group of cystine are typical of the corresponding alkyl sulfur compounds. The hydroxyl group of tyrosine shows the color and chemical reactions of simple phenols, while the functional groups of histidine, arginine, methionine, and tryptophan resemble those of compounds not so familiar but forming well-understood chemical families. Reactions of the purely aliphatic hydrocarbon structure exist but are generally of little value since the conditions necessary for reaction are usually so drastic as to break up the protein itself.

As expected from the properties of peptides, proteins may be hydrolyzed to smaller fragments, depending on the conditions and duration of treatment. Peptide bonds may be split by acids, alkalis, or certain enzymes known as proteolytic enzymes. Hydrolyses may open only one or all of the peptide bonds and serve in laboratory investigations, digestion of food proteins, or various transformations inside cells.

Proteins undergo one type of reaction, probably partly chemical and partly physical, that is relatively peculiar to this group. When heated, most proteins undergo irreversible changes in solubility and molecular shape. Apparently a number of bonds are broken, at least some of them being hydrogen bonds. When soluble, globular proteins are heated, they become insoluble, and the molecules elongate or unfold to resemble fibrous proteins. These changes are not well understood but are widely encountered and are called denaturation or sometimes coagulation when a curdling or extensive clumping results. Denaturation often accompanies chemical alteration, including hydrolysis of proteins. In fact, enzymatic hydrolysis of proteins may require denaturation as the first step before any peptide bonds are split. At any rate heat denaturation (cooking) alters the digestibility of our food proteins.

The physical properties of proteins differ markedly in some respects but not in all. Many proteins are very soluble in water, many are not, and some are intermediate. None dissolve in non-polar solvents, and only the prolamines are soluble in even moderate concentrations of ethanol. Proteins are always solids. They cannot be heated remotely near to melting without complete decomposition.

Protein molecules vary in size by a factor of about a thousand, and they vary in shape from spheres to relatively linear peptide chains. The latter type is usually not soluble without decomposition, and bundles or sheets of such long molecules make up hair, horn, membranes, and finger nails. Soluble proteins may often be crystallized, the molecules fitting into regular arrays like those formed by common smaller molecules. These soluble proteins are often termed globular, though they may be ellipsoidal, that is, with elliptical cross section. In fact, some natural forms are so exaggerated as to be of a cigar-like shape. However, all these probably have their peptide chains more or less looped back and forth instead of stretched into a single filament.

Actually, the bond angles of the atoms, the bond lengths, and the sizes of the groups attached require that the peptide chain be a rather complex affair. It has been proposed that this structure corresponds to a helix with the side groups outside the spiral core of the peptide chain. This configuration is believed to be stabilized by hydrogen bonds between carbonyl and amide —NH— groups on different turns of the spiral. How such a helix is folded to fit into a roughly globular pattern is still unknown.

As dipolar ions, proteins react with both acids and bases, accepting or giving up protons. Such reactions make them buffers resisting changes in pH. Moreover, proteins exhibit characteristic isoelectric points, depending upon their natures and their environments. They undergo electrophoresis and may be separated by means of this phenomenon when they migrate through the electrical field at different rates.

Proteins are enough alike chemically to undergo certain general reactions. One of these is a test for the presence of two or more adjacent peptide bonds and is called the biuret test. It is based on the fact that a violet color appears when a protein or tripeptide is treated with sodium hydroxide and dilute copper sulfate. The color arises from the formation of a complex of cupric ion with two peptide bonds or one peptide bond and one of several other kinds of structures. The color is similar to that formed between copper and biuret, $H_2NCONHCONH_2$, giving the test its name.

Heating a protein with nitric acid produces a yellow color that turns orange on addition of alkali. Aromatic amino acids, especially tyrosine and tryptophan, are responsible for this test, known as the xanthoproteic reaction. Many phenols yield red colors or precipitates when treated with an acid solution of mercuric, nitrous, and nitrate ions. This reaction is called Millon's test and is given by proteins by virtue of the tyrosine normally present.

Biological Properties

Proteins serve in many biological roles. Most of these are critical to life or at least to certain forms of life. Yet the diversity and complexity of function are so great as to preclude extensive discussion here. Some of these roles are outlined later, as noted below.

Many proteins display catalytic activities, accelerating reactions to rates great enough for the needs of living cells. Life appears to depend upon continuously and rapidly available supplies of many substances. Thus cells require a large variety of effective reaction catalysts, and as a result they contain many different enzymes. The particular properties of enzymes are discussed separately in Chapter 6.

Proteins perform special transport functions. Many compounds penetrate cells and accumulate inside at much higher concentrations than expected from diffusion alone. These transfers require the input of energy needed to produce a higher concentration inside than outside the cell and are usually termed active transport. The mechanism of active transport is still not clear, but it does involve proteins either as catalysts or as adsorbents or both. In some cases of active transport, enzymes convert a substrate to some other derivative which may then pass through the cell membranes. However, if only free diffusion controlled the passage, the derivative ought to diffuse out into the environment at the same time. Usually such products are not found outside the cells, so perhaps they are adsorbed on proteins too large to escape from the cell and accumulate inside in the form of protein complexes.

In higher animals there are circulatory systems which basically are colloidal systems containing suspended cells in many species but without cells in other species. In either case, the respiratory pigments present form complexes with oxygen in regions like the lungs where the partial pressure of the gas is high. These complexes circulate in the blood to organs where the demand for oxygen has reduced the partial pressure. Satisfaction of the equilibrium constant then leads to dissociation of the complex and a supply of oxygen for the tissues utilizing it. Other gases, including the toxic carbon monoxide, may be transported in the same way. Likewise, non-gaseous solutes appear to be transported as adsorption complexes with blood proteins, particularly with serum albumin. Certain vitamins, for example, associate with serum albumin as blood circulates past the digestive tract. Dissociation of these complexes then supplies required vitamins to those cells not directly in contact with the food supply.

Proteins serve as fibrous components of the tissues holding the rigid skeletal elements together. Collagen is the fundamental material of connective tissue; it serves primarily in a structural role. Muscle, also largely protein, fills structural needs, but its principal function is mechanical, being the tissue in which chemical energy is converted into mechanical energy necessary for physical activity.

Certain of the nucleoproteins serve as carriers of genetic traits and appear to control hereditary characteristics. The mechanism of this control is of intense interest but so far has not been elucidated. Related nucleoproteins called viruses are the infectious agents inducing many common diseases in plants and animals.

Animals are characterized in part by their extensive nutritional requirements and have less synthetic ability than do green plants and microorganisms. Proteins or at least certain essential amino acids and perhaps peptides must be available for the well being of animals. Protein metabolism and nutrition are discussed on page 463 along with requirements for particular amino acids.

The velocities of biological processes are subject to indirect controls of specific types in at least the moderately complex forms. The substances involved act mainly on the enzymes catalyzing the reactions affected and are called hormones. Hormones of several chemically different classes, including proteins and peptides, are known. They are considered later in connection with the regulation of the growth of plants and of various physiological functions of animals.

Finally, proteins are known to be toxic to animals under particular conditions. Usually toxicity arises only when proteins are transferred from one species to another, without opportunity for denaturation or digestion. Venoms of snakes and insects are injected by biting into the tissues or circulatory systems. Certain of these foreign proteins are actually enzymes that attack components of the body, producing disturbances that may result in death. Destruction of blood cells and proteins is typical of the action of the proteins in snake venoms. In other cases, as with certain bacterial toxins, the proteins may prevent the function of special nerve circuits. The toxin from *Clostridium botulinum* blocks the action of the nerve of the diaphragm when injected into a mammal.

Other toxicities involving foreign proteins result from responses by the affected animal. Allergic reactions fall into this category and occur when an animal is exposed to a foreign protein to which it has been sensitized by prior exposure. The extent and severity of the symptoms depend upon the nature of the foreign protein, the mode of administration, the quantity given, and the animal species concerned. The toxic disturbances range from respiratory congestion, skin blisters, and swelling of limbs to unconsciousness or death. The symptoms depend upon physiological changes induced by the original sensitizing exposure and occur on re-exposure to the same foreign protein. Owing to its medical importance, this problem has been intensively studied, but the mechanism of the process is still unknown.

NUCLEIC ACIDS AND NUCLEOPROTEINS

Nucleoproteins

These natural macromolecules are made up of two parts. One part is protein and the other nucleic acid. The two components are attached, but the nature of the bonds is not fully understood. The nucleic acids, as the name suggests, are acidic compounds and exist as anions at physiological pH values. On the other hand, the protein components of the nucleoproteins seem to be of the histone type and carry net positive charges. Thus salt formation between the nucleic acid and protein is a strong possibility, but there may be other forces of combination as well. At any rate, though it is common to speak of and study nucleic acids by themselves, it is probable that they do not exist independently in cells. Rather, the nucleic acids are associated with proteins in their natural state.

The chemistry of the nucleic acids and their components is outlined below, but even less is known of the chemistry of the associated proteins than of proteins in general. Isolations are difficult and careful fractionation studies lacking, so that it is not clear whether the nucleoproteins from a single source are based on one or on a multiplicity of proteins. Probably there are many different protein components since there are believed to be many different nucleic acids and nucleoproteins. The histone (or histones) involved does have an exceptionally high content of the basic amino acids arginine and lysine.

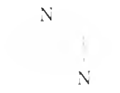
Nucleoproteins may be regarded as aggregates of successively smaller subunits. These various levels of composition may be classified as follows, proceeding in the direction of the smallest components:

Nucleoproteins
↓
Nucleic acids + Proteins
↓
Nucleotides
↓
Nucleosides + Phosphate
↓
Ribose or deoxyribose + Pyrimidine or purine bases

Nucleotides

These compounds consist of organic bases and phosphoric acid condensed with particular pentoses. The sugars known to be involved in some nucleic acids are ribose and deoxyribose (see page 62), although others may be found. In addition, glucose, galactose, and other sugars occur in special nucleotides of metabolic importance but have not been isolated from nucleic acids.

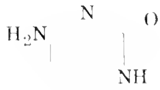
The bases belong to two series, derivatives of pyrimidine and purine. These are:



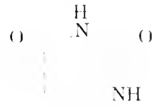
pyrimidine

purine

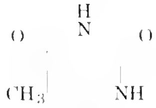
The four pyrimidines most abundant in nucleic acids are:



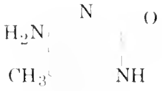
cytosine



uracil

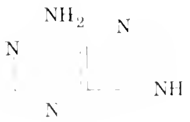


thymine

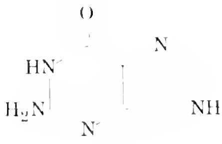


5-methylcytosine

One other, hydroxymethylcytosine, has been isolated in the nucleic acids from certain bacterial viruses. The most abundant purines are:

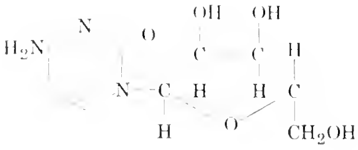


adenine

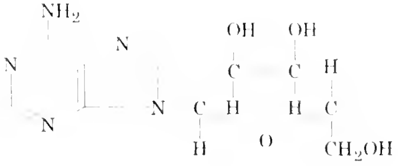


guanine

with others known from metabolic processes. These bases are condensed by way of the semiacetal forms of D-ribose and D-deoxyribose to form ribonucleosides and deoxyribonucleosides, respectively. Not all possible combinations are known. Cytosine, uracil, adenine, and guanine are found attached to ribose and cytosine, methylcytosine, hydroxymethylcytosine, thymine, adenine, and guanine with the deoxy sugar. The linkages are similar for all pyrimidines on the one hand and for the purines on the other. Thus, the structures of the nucleosides may be illustrated by:

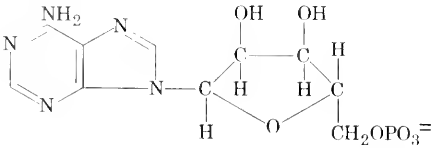


cytidine



adenosine

These nucleosides are esterified with phosphoric acid in the nucleotides. Any of the available hydroxyl groups could participate and do, at least under special conditions. In the nucleic acids, however, the ester bonds probably involve only the hydroxyl groups of carbons three and five of either ribose or deoxyribose. Adenylic acid is a typical nucleotide. The nomenclature of all these related compounds may be summarized thus:



adenosine-5′-phosphate
adenylic acid (from muscle)

Base	Nucleoside	Nucleotide
Cytosine	Cytidine	Cytidylic acid
Uracil	Uridine	Uridylic acid
Thymine	Thymidine	Thymidylic acid
Adenine	Adenosine	Adenylic acid
Guanine	Guanosine	Guanylic acid

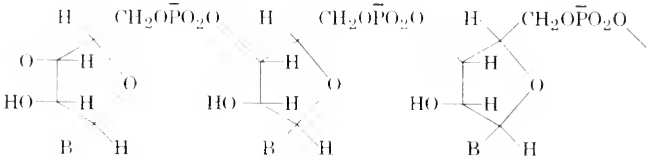
These and the other closely related nucleotides make up the nucleic acids. In addition, some of them have exceedingly important independent metabolic roles, one being the transfer of chemical energy. Moreover, there are other similar nucleotides performing related metabolic functions. These important considerations are developed in later chapters.

Nucleic Acids

The natural condensation products of nucleotides reach macromolecular dimensions, are called nucleic acids, and are found in all ordinary cells. They are associated in turn with protein in nucleoproteins. Although much is still unknown, a start has been made toward understanding the structures of the nucleic acids.

In the first place these compounds fall into two groups, those containing ribose and those containing deoxyribose. These classes are known, respectively, as ribonucleic acids or RNA and deoxyribonucleic acids or DNA. When the sugar has not been positively identified, it is customary to use the terms pentosenucleic and deoxypentosenucleic acids, just in case the sugar turns out to be another pentose. So far as is known, each nucleic acid contains but a single kind of sugar, either D-ribose or D-deoxyribose.

Both types of nucleic acids appear to be chains with alternating sugar and phosphate groups. The purine and pyrimidine bases are distributed along this chain, one for each sugar residue. The chain itself depends upon ester bonds between phosphates and the adjacent sugars with the hydroxyl groups of positions three and five involved as follows:



where B represents cytosine, uracil, adenine, or guanine. The indicated negative charges arise by loss of protons from the phosphoric acid groups and are present at physiological values of pH. Only strong acids can restore the proton, converting the salt form into the acid. Thus the nucleic acids normally occur as salts rather than as true acids.

As shown above, it is now believed that the second hydroxyl group of ribose is free. Formerly the ribonucleic acids were thought to be branched with the branches attached at position two. Present evidence, however, suggests that branching does not occur.

On the basis of X-ray data it has been postulated that this sugar phosphate chain is arranged in a spiral with the bases fitted inside this spiral. This idea is somewhat analogous to that for the peptide chains of proteins. The sequence of the bases is just beginning to yield to present methods of study. It is known that the four bases in the ribonucleic acids occur in different relative amounts, and hydrolysis studies show that the distribution is not uniform. Rather, there are regions of the molecule containing only the pyrimidines, others only purines, and others with the two types at more or less alternating sites.

It is assumed that cells may contain ribonucleic acids of different types. Certainly different ones can be isolated from different organs and animals. However, sensitive biological tests for different ribonucleic acids have not been found. Thus it is not possible to perform sensitive fractionation studies. In addition, the ribonucleic acids are very sensitive to degradation processes when in solution. This complicates the isolations, so that, even when separations are achieved, the fractions obtained may not represent the structures originally present in the source materials.

The biological roles of the ribonucleic acids are still not clear. It is known that the synthesis of proteins by cells is accompanied by synthesis of ribonucleic acid. It has been reported that, if protein synthesis is blocked, synthesis of ribonucleic acid can still occur. But apparently the converse is not true, since blocking the synthesis of ribonucleic acid blocks that of protein too. Hence it is believed that ribonucleic acid is somehow involved in protein formation, but there is as yet no conclusive support for any mechanism proposed.

Much of the above structural information applies equally well to the deoxyribonucleic acids. In these compounds the second hydroxyl group of the sugar is replaced by a hydrogen atom. This difference reduces the opportunity for branching of the chain, and there is no evidence of branches. A spiral form is assigned to the sugar phosphate chain with the bases distributed as in the related compounds. However, at least some of the deoxyribonucleic acids contain more than four different bases. Adenine and guanine are the only known purines, but cytosine, thymine, 5-methylcytosine, and 5-hydroxymethylcytosine are all known to occur, though perhaps not all in the nucleic acid from any single source.

The problems of isolation and separation again are difficult and complicated by the instability of these macromolecules, which are nevertheless more stable than those of the ribonucleic acids. Although sensitive biological tests have been devised, they are still of limited application and so far have contributed little to the general structural study of deoxyribonucleic acids.

Extensive investigation assigns the deoxyribonucleic acids, through their nucleoproteins, a primary role in heredity. It is now believed that the structures of these compounds contain information chemically transmitted to daughter cells and fixing the character of these cells. Since detailed structural information is still largely lacking, the current theories of mechanism have no real basis except perhaps that of possibility. Certainly the deoxyribonucleic acids are important, since alteration of these compounds inside cells leads to inability to multiply or to death.

The infectious agents of the virus group also contain nucleic acids, sometimes of the ribose and sometimes of the deoxyribose type. Viruses specifically attack particular host cells and appear to be innocuous for all species outside their individual host ranges. Well-known diseases are produced in all groups of the animal kingdom, including mammals, of course. Plant viruses may be equally widespread and certainly are of great agricultural importance. Even microorganisms are parasitized by viruses. Much study has been devoted to those types of viruses infecting bacteria in the expectation that information gained for these forms, called bacteriophages, might be applied to the viruses of plants and animals.

Viruses are not typical cells. No respiration can be demonstrated for them, and none of the enzymes of carbohydrate, lipide, and amino acid metabolism have been found. Furthermore, they do not reproduce independently, but increase in numbers only inside the host cells. Apparently infection by a virus particle somehow induces the host cell to cease some of its normal functions and synthesize components for new virus. Some portion of the original virus particle then controls the assembly of the components, thus resulting in virus production. Death of the infected cell frees the new particles and permits spread of the infection.

Obviously much has been learned about nucleic acids, yet work in the field is unusually difficult and far more remains unknown than has been clarified. One of the great lacks is that of sensitive tests for nucleic acids of different kinds.

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Enzymes

6

NATURE AND FUNCTION

Enzymes are proteins with catalytic activities. When the definition of proteins from page 111 is inserted in the sentence above, enzymes are defined as macromolecules of biological origin consisting largely of amino acids condensed via peptide bonds and capable of altering the rates of reactions. Thus the chemical and physical properties of enzymes are those of proteins in general, excepting of course, the catalytic function. Hence it is not necessary to discuss further the basic structure of enzymes. Rather the present chapter will be devoted to enzymes as catalysts.

It happens that no other types of naturally occurring macromolecular catalysts are now known. Perhaps some will ultimately be discovered, but the enzymes are clearly fundamental units in the control of biological reactions. In fact, some workers feel that the term *enzyme* should be applied to any and all conceivable natural macromolecular catalysts without regard to their chemical constitution. This conception hardly seems warranted of chemists or biochemists who traditionally classify molecular types on chemical bases. If some large non-protein catalysts are demonstrated, they would be more properly grouped with chemically related compounds than with proteins. Nowadays whenever a giant molecule is found that accelerates a reaction, it is customary to call it an enzyme, all too often without the demonstration of protein properties. Such an assumption may well be valid but can hardly be justified on the sole possible basis of a general rule derived only from past experience.

There are many known catalysts consisting of large particles, though apparently none of these except proteins function in biological systems. Thus these non-biological particulate or colloidal catalysts are differentiated from enzymes by means of their origins. Ultimately the synthesis of enzymes will be an accomplished fact. Probably synthetic proteins with new kinds of catalytic activities will be made as well. Such presently foreseeable materials will undoubtedly be classed with the natural enzymes because of their structural relationship. This grouping will require a slight change in the definition of enzymes.

Classification

As was the case with proteins in general, the classification of enzymes is in a rather unsatisfactory state. Many schemes have been used for diverse purposes, but as might be expected the groups often overlap. Moreover, the properties classified in one system seldom bear much of a relationship to other important properties. Even in this unsatisfactory state, an extensive discussion of enzyme classification is desirable because it provides coverage of much of the terminology widely used in enzyme work.

As relatively typical proteins, enzymes may be classified according to the various systems used for proteins. Whenever source is considered to be of basic importance, it is customary to speak of the enzymes of plants, bacteria, the red blood cell, and mitochondria (particles containing several enzymes and isolated from a variety of cells). Many enzymes contain only amino acids and hence are simple proteins and represent several solubility groups. Others possess additional structures, thus become conjugated proteins, and are subdivided in the same way as the non-catalytic complex proteins.

Recently enzymes have been classified according to the conditions governing their occurrence in cells. Many enzymes seem to be rather standard equipment in the cells of a given species and have been called constitutive enzymes. Others exist in low concentrations or perhaps do not occur at all in particular environments. Yet when certain critical substances occur in the environment, an enzyme is formed seemingly to meet the need thus arising. These specially formed proteins are known as adaptive or inducible enzymes. Once it was thought that constitutive and inducible enzymes differed in some unknown fundamental manner involving the biological control of their formations. But evidence is appearing that suggests there may be no major differences. Probably this classification will ultimately disappear.

Some workers have studied the biological stability of enzymes under adverse conditions. Certain enzymes seem to be retained without much diminution by starving cells. Others decrease drastically long before the cells themselves die. Thus the organisms exist at least temporarily without a full complement of these latter dispensable enzymes. Although the persistent group may or may not actually be critical to the cells, they seemingly cannot be broken down to any great extent. This discovery may apply to other proteins as well as enzymes and be a general phenomenon. Or it may transpire that inducible and dispensable enzymes are basically the same, appearing and disappearing as the critical molecules are added to or disappear from the environment.

Work on the mechanism of food digestion, the transport of substances across cell walls, and the like has led to the classification of enzymes according to the site of action. The two major groups are intracellular and extracellular enzymes, respectively, occurring and functioning inside or outside the cells synthesizing them. In addition, enzymes of the cell surface are occasionally considered separately.

All the above classifications have important applications but mostly for rather specialized purposes. However, enzymes as a group are most noted for their catalytic function. Since they are distinguished from proteins in this particular regard, a natural system of classification might be based on differences in catalytic action.

Several possibilities exist for classification along this general line. The mechanism of enzyme action might be chosen, for example. However, as yet too little is known to make this basis a useful one, although ultimately it may become important. Secondly, enzymes might be grouped according to the extent of their catalytic effects, in other words according to the degree to which they accelerate the reactions affected. This procedure would automatically require comparisons with equal quantities of other enzymes, requiring in turn knowledge of the concentration and purity of the enzymes investigated. Since the purity of proteins and enzymes is difficult to ascertain, this second basis of classification is seldom used.

Finally, enzymes may be classified according to the kinds of other compounds involved in the reactions, either the reactants or the products. Usually the reactants are chosen for the purpose. When more than one kind of molecule is involved, only one is selected in order to simplify the nomenclature. The choice seems to be a matter of custom, convenience, or personal preference. In a number of cases the names of classes of enzymes denote the type of reaction catalyzed instead of the reactants. Depending upon the circumstances of classification, a particular enzyme may fall into one category or another or perhaps into two. For example, pepsin catalyzes the partial hydrolysis of proteins. It is called a protease or proteolytic enzyme or more generally a hydrolase, a class including all enzymes catalyzing hydrolyses. Pepsin also splits certain peptides and thus could be called a peptidase. Thus it becomes apparent that the classification of enzymes is not completely standardized and may be adapted to the needs of the user.

In enzyme chemistry one of the reactants is always of particular interest for reasons of structure, occurrence, or ultimate biological utilization. This reactant is normally referred to as the substrate of the enzyme in question and is a principal focus of attention. Again in the case of pepsin, a protein and water react during the enzymatic process. The water is very abundant in cells, of course, and is of less specific interest in work on this reaction system than is the protein. Hence the protein is known as the substrate of pepsin, although the water is clearly a fundamental necessity in the reaction.

The general theory of catalysis states that catalysts merely alter reaction rates but never initiate reactions that would not occur in the absence of the catalyst. In practice this concept is difficult to prove or even apply because many reactions go so slowly that products cannot be detected until a catalyst is added. The reaction

$$2H_2 + O_2 \rightarrow 2H_2O + \text{energy}$$

is theoretically a spontaneous one with its equilibrium position at room temperature far in the direction of water. Yet oxygen and hydrogen apparently can exist together indefinitely until a catalyst is introduced. In cases of this kind including a number of biological reactions, it is difficult to decide whether a catalyst initiated a reaction or merely accelerated it enormously. Possibly concern over this problem is akin to tilting with windmills, but only time and knowledge can decide.

On the other hand, there are many catalyzed reactions that do proceed at slow but spontaneous rates. In these examples, there can be no question of initiation of reaction since the instant reactants are combined the process starts. Perhaps the catalyst could initiate the reaction, but it never does if the reactants are mixed before the catalyst is added. When one reactant is combined with the catalyst, addition of the second reactant starts the process. Since reaction would begin without the catalyst, it seems impossible to attribute any triggering action to the catalyst itself.

As mentioned on page 113, enzymes are commonly denoted by the suffix *ase* except for a few long-established names for some of the hydrolytic enzymes and for two oxidation catalysts still called the yellow enzymes. The roots of names for enzymes are usually taken from the substrate of major interest and often consist of two (sometimes three) words, one designating the substrate, the other specifying the change produced in the substrate. For example, α-amylase catalyzes the hydrolysis of the α-amylose component of starch, and alcohol dehydrogenase catalyzes a particular dehydrogenation of ethanol. There are a few special cases not named from the substrate but having the typical ending. Invertase promotes the conversion of sucrose to a mixture of D-glucose and D-fructose. The name refers to both the inversion of optical rotation that takes place and to the products in this mixture called invert sugar.

TABLE 6–1. Classification of Enzymes

Enzymes	Substrates or Reactions
Hydrolytic enzymes	Hydrolysis
Proteases (proteolytic enzymes)	Proteins, peptides
Esterases and lipases	Esters, lipides
Carbohydrases (glycosidases)	Glycosides (acetals)
Phosphatases	Phosphate esters and anhydrides
Amidases	Amides
Peptidases	Small peptides
Oxidation-reduction enzymes	Oxidation and reduction
Oxidases	Oxidations with O_2 or H_2O_2, decomposition of H_2O_2
Dehydrogenases	Transfer of hydrogen atoms to or from substrates
Transferring enzymes	Exchange of groups between substrates
Transphosphorylases	Phosphate
Transglycosidases	Carbohydrate
Transpeptidases	Amide, amino acid
Transaminases	Amino
Transmethylases	Methyl
Transacylases	Acyl
Addition enzymes	Addition of H_2O or NH_3 to carbon-carbon double bonds
Isomerizing enzymes	Change of configuration about a carbon atom or other rearrangements in a substrate molecule
Carboxylation enzymes	
Decarboxylases, carboxylases	Elimination or addition of CO_2

Enzymes are commonly assigned to one of a series of groups based on the type of reaction concerned. The number and definitions of these groups vary with the interests of the authors concerned. The subdivision of the major groups is based on the relationships of the substrates involved. The scheme of Table 6–1 represents one classification of enzymes.

A number of these classes contain many different individual enzymes and may be further subdivided. The oxidases, for example, may be grouped into the oxidases containing iron, those containing copper, and those lacking an attached metal. This procedure introduces a new type of classification, according to the nature of the special attached or associated structures. Thus, certain of the oxidases are metal enzymes, specifically either iron or copper enzymes.

In the above cases the metal is rather firmly bound to the protein and is not removed by dialysis under physiological conditions. Groups thus firmly bound are called prosthetic groups. Besides metals there are other types of prosthetic groups, including phosphoric esters and complex organic structures called porphyrins, usually associated with iron in enzymes. In fact all the non-amino acid components of the conjugated or complex proteins may be termed prosthetic groups, although not all types are represented among the enzymes themselves.

The same or similar prosthetic groups are attached to quite different protein components or moieties. Since the resulting enzymes differ in their activities, the catalytic properties of an enzyme of this type depend on both the prosthetic group and the protein moiety.

In other enzyme systems associated rather than attached structures are important for catalytic action. These associated materials are rather readily separated from the enzyme themselves. They are called cofactors and are discussed more extensively on page 139.

Catalytic Action

Enzymes are distinguished from other proteins by their catalytic action, and this property is the most important one enzymes have. The diversity of reactions catalyzed is known to be great (see page 130), and our knowledge is still increasing. Since enzymes function biologically, most of the reactions involve organic compounds, at least in part. A few enzymes catalyze the reaction of inorganic substrates, as in the following:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$
$$2H + H_3O^+ + SO_4^= \rightarrow HSO_3^- + 2H_2O$$
$$2H + NO_3^- \rightarrow NO_2^- + H_2O$$

where 2H stands for a particular reducing agent, depending on the enzyme. A diversity of catalyzed processes is a fundamental requirement of biological systems. Without the enzymes, reaction rates would be too slow to allow the existence of life as we know it.

Enzyme activity depends upon many factors. For example, a suitable substrate must be present. This problem is referred to as enzyme specificity or substrate specificity and is discussed in the following section. With a given enzyme-substrate system, the reaction rate depends upon the concentrations of enzyme, reactants, and products, the presence of cofactors, the presence of inhibitors, the temperature, the pH, and the solvent. These special factors are discussed in a separate division beginning on page 137.

Specificity

The specificity of enzyme action is suggested by the classification system applied to enzymes in Table 6–1. There the enzymes were grouped according to reaction types or classes of substrates. In either case, the assumption is implicit that an enzyme placed in any category does not directly affect the reactions catalyzed by enzymes from any other category. For a long time this assumption was believed immutable, but a few enzymes are now known to promote changes in substrates of different types when studied *in vitro*. As an example, chymotrypsin, formed in the pancreas and important in digestion, catalyzes the hydrolysis of peptide and amide bonds in proteins and peptides. In addition, chymotrypsin acts as a transpeptidase, transferring amino acids from one peptide to another. Moreover, this same enzyme catalyzes the hydrolysis of certain synthetic ethyl esters. Thus chymotrypsin could be placed among the proteases, transpeptidases, or esterases. Ordinarily it is placed in the first class since it definitely functions in proteolysis in animals. The biological importance of the last two types of activity is still in doubt.

Although some other enzymes also can perform dual functions, the number known is still quite small, helping to simplify problems of classification and specificity. Eventually additional activities may be discovered for many enzymes, but there is no simple systematic means of searching for all the functions of enzymes. Therefore, until more or less accidental discoveries bring many additional activities to light, it is easiest to recognize the problem and solve it by learning the known individual exceptions.

There are widely differing degrees of specificity. Pepsin splits most proteins except the fibrous types into peptides. It attacks some polypeptides and a few known small peptides. Thus this enzyme is specific for peptide bonds (as far as is known), but these peptide bonds must involve certain definite amino acids. Nevertheless, a wide variety of different substrate molecules is attacked. At the other extreme, urease catalyzes only one known reaction,

$$H_2NCONH_2 + H_2O \rightarrow 2NH_3 + H_2CO_3$$

Such absolute specificity as this is relatively rare. Usually the degree is somewhere intermediate between these two extremes. It is common for an enzyme to catalyze the reaction of one or at most a few substrates in living systems. In the test tube, this enzyme can promote reaction of synthetic substrates closely related to the naturally occurring one. For example, catalase decomposes hydrogen peroxide and presumably serves to prevent accumulation of toxic concentrations of this product of the action of certain oxidases. Catalase accelerates the breakdown of ethyl hydrogen peroxide, C_2H_5OOH, a compound unknown and unlikely in biological sources. Structurally related compounds may thus often be used as substrates for an enzyme.

Stereochemical specificity is widely encountered. One optical isomer serves as a substrate; the other is completely inert. A peptidase, carboxypeptidase, hydrolyzes the synthetic peptide derivative carbobenzoxyglycyl-L-phenylalanine but has no effect on the D isomer. Maltase hydrolyzes maltose and several other α-glucosides but no β-glucosides. β-Glucosidase splits the β but not the α isomers. D-Amino acid oxidase is widely distributed in animal cells and is specific for the D forms. The less common L-amino acid oxidases occur in quantity in snake venoms and are equally specific. This high degree of optical specificity is commonplace and is important, since cells seem to make a preferred optical form whenever two or more forms of metabolic compounds are possible. Indeed it is suspected that the optical specificity of enzymes may be primarily responsible for the occurrence of optical specificity among the other biological molecules.

When related substrates are attacked, as by catalase in the example given, the rates often differ. Sometimes the natural substrate reacts more rapidly, sometimes less rapidly, than the synthetic substances. The reasons for these differences are obscure but probably lie in the chemical reactivities of the substrate molecules and in the matching of the geometry and force fields of substrate and enzyme. It seems likely that enzymatic catalysis always involves the formation of a complex between enzyme and substrate. If the structures of the enzyme and substrate do not properly correspond, they cannot fit together to form a reactive complex. When the correspondence is not perfect, catalysis may be possible but less efficient. Thus the presence of groups not directly involved in the reaction can affect the rate or even prevent reaction. Whenever the side groups alter the reactivity of a molecule, they will also alter the efficiency of the enzyme, and the rate will be changed.

Currently, investigators believe that only portions of an enzyme molecule participate directly in the catalytic action. The nature and spacing of the directly important groups determine the specificity of the enzyme. When more is learned of the general requirements for enzymatic activity, it may become possible to predict substrate function after some knowledge of enzyme structure has been obtained.

Biosynthesis

The word *biosynthesis* refers to the method of synthesis employed by cells. In the case of proteins and enzymes the mechanism of biosynthesis is one of the greatest problems of biochemistry. Understanding is necessary for that ultimate goal, the complete synthesis of a living cell. Even now the development of concepts of enzyme synthesis has led to theories of the origin and evolution of life.

Much of the work on the biological formation of proteins has depended on study of the synthesis of enzymes. This emphasis on the latter arises from the biological label inherent in the catalytic activity of enzymes, making them relatively easy to detect and measure. Moreover, the adaptive or inducible enzymes are natural subjects for study because their formation can be initiated or at least accelerated by suitably modifying the cellular environment. Yet in spite of this important starting point, only a few general ideas have been worked out. The difficulties stem largely from the size and complexity of protein molecules and from lack of knowledge of the nucleic acids. Since most of our present information has been obtained in the last decade, the next should provide great progress.

Enzyme formation in particular and protein synthesis in general obviously depend upon the presence of a supply of amino acids. These components of the protein are then combined to form the resulting specific macromolecule. There are several major problems involved. Are the amino acids combined one after another, forming a peptide that grows into protein? How is the synthesis controlled to give a specific protein structure and not a mixture of all possible structures? The energy content of a peptide or protein is higher than that of a mixture of the individual amino acid components. Where does this energy come from and how is it transferred during the formation of the peptide bonds? Are catalysts involved in the synthesis, and if so what are they? All these problems lead to smaller problems and ramifications of their own.

At present the matter of timing in the combination of the component amino acids is not established. Some experimental results suggest that intermediate peptides do not appear. All the peptide bonds must be formed practically simultaneously. Moreover, when a compound is added to the system preventing incorporation of one kind of amino acid into enzyme, none of the other amino acids are combined. On the other hand, experiments with different cells and enzymes and by different investigators contradict the above interpretation. Although it has been assumed that different organisms synthesize their proteins in the same way, perhaps they do not. Or maybe different enzymes arise by unrelated routes.

Ribonuclease catalyzes the partial hydrolysis of ribonucleic acids into the individual nucleotides. This widely distributed enzyme has been isolated and extensively studied to ascertain the chemical and physical similarity of the enzymatically active individual molecules. Ribonuclease from a single source can be separated into two fractions, one of them small in quantity, both with catalytic activity. Perhaps the one fraction is an artifact of isolation. At any rate, incomplete structure studies on the predominant fraction make it most probable that all the molecules of this particular principal enzyme fraction are alike, even to identical sequences of amino acids in the peptide chain. Thus there are highly preferred structures for enzymes, although there may occasionally be more than one kind.

How can such preference be expressed during enzyme biosynthesis? Some organisms can make a particular enzyme and some cannot. Experiments involving both enzyme synthesis and genetics show that the former is under hereditary control. Special techniques modifying the heredity of a cell can remove or introduce the capacity for the formation of one of a variety of enzymes. This relationship then ties the biosynthesis of enzymes to hereditary in general. More specifically, the use of inhibitors acting on the nucleic acids blocks all enzyme synthesis. In addition, a cell deficient in the ability to make a particular enzyme gains this ability when properly treated with deoxyribonucleic acid extracted from cells related to the deficient cell but possessing the synthetic capacity missing from such cells. Hence this type of nuclcic acid is important in the control of enzyme synthesis. Moreover, similar experiments make deoxyribonucleic acid the basis of heredity in general.

Although required, deoxyribonucleic acid is not the only essential factor. The ribonucleic acids must also be involved. Ribonuclease acts only on ribonucleic acid. Systems treated with this enzyme lose their ability to synthesize other enzymes. When radioactive isotopes are used to follow the formation of an enzyme, ribonucleic acids are also formed. Anything blocking the synthesis of ribonucleic acids blocks enzyme synthesis.

The current hypothesis concerning the control of enzyme synthesis runs as follows. Deoxyribonucleic acids carry the hereditary chemical information necessary for precise specification of enzyme structure from mother cell to daughter, thus providing inheritance. The information thus available is somehow transferred through ribonucleic acid into enzyme structure with ribonucleic acid and protein synthesized at about the same time.

It is not yet known whether the nucleic acids serve a catalytic role, take part in energy transfers, or function only as patterns or templates. Clearly they are involved somehow, almost certainly in the last capacity but perhaps in others also.

Precursors

In several cases enzymes are synthesized and secreted in inactive forms called precursors. These proteins are not really enzymes, of course, because they display no catalytic activity but do give rise to enzymes under special conditions. It is difficult to see why cells should make precursors for some enzymes and not others, rather than merely forming the active enzymes in all cases. Perhaps the precursors are evolutionary anachronisms. Or perhaps they are required at times or places when the active enzymes would injure cells. Many of the known precursor proteins convert into proteolytic enzymes which might conceivably cause the destruction of essential protein components in the cells synthesizing these specialized materials.

Pepsinogen is a well-known precursor system. It is secreted by the giant or chief cells into the stomach, where the acid secreted by other cells helps convert the precursor into active pepsin. Any pepsin already present catalyzes this conversion, which involves splitting off a peptide fragment. The molecular weights of the various molecules are as follows: pepsinogen, 42,000; pepsin, 36,000; peptide, 6,000.

$$\text{Pepsinogen} \xrightarrow[\text{pepsin}]{H_3O^+} \text{pepsin} + \text{peptide}$$

Several of the enzyme-precursor systems are important in the digestive processes of mammals and birds. Others are believed to participate in the disintegration, called autolysis, of dead cells. Still other systems of this type seem to play roles in the metamorphosis of insects.

FACTORS AFFECTING ENZYME ACTIVITY

The rate of reaction in an enzyme-substrate system depends first of all on the natures of both the reactants and the catalyst. The fundamental basis of this dependence is certainly chemical, at least in part, but there may be important physical factors like size. Although the problem has been given much consideration, little has been learned.

Aside from this innate dependency, the nature of the environment in which the system exists has great influence. In this case the experimentalist has a better opportunity for understanding. He can alter the environment almost at will and observe the effect produced.

Solvent

The solvent is important, first because many enzymes are active only in solution (colloidal dispersion) and dissolve only in water. Others act while in the solid state in the particulates of cells, but the substrates are soluble only in water. All enzymes seem to require the presence of water for catalytic activity. Indeed water is either a reactant or a product in a great many enzymatic reactions. The addition of solvents miscible with water may retard the rate of reaction at moderate concentrations but not stop it until the concentration becomes high.

Added inert, uncharged solutes have effects similar to those of the liquids above, slight at low concentration, important at high concentration. When salts are added, the resulting changes in ionic strength may modify the reaction rate sharply. Some enzyme systems are quite sensitive to changes in ionic strength, but others are not. Actual behavior depends upon the effect of ionic strength on the structures of the enzyme and substrate and perhaps on the complex between them. Little is known on this point.

Concentrations of Enzyme and Substrate

Enzyme-catalyzed reactions may be represented by

$$\text{Reactants} + \text{enzyme} \leftrightarrows \text{complex} \leftrightarrows \text{products} + \text{enzyme}$$

This overall process may involve more than one reactant or substrate and more than one kind of intermediate complex. Moreover, in an equilibrium system such as this, the concentrations of the products affect the reaction rates. Usually it is customary to simplify consideration of the rates by examining the reaction under special conditions. In the first place, attention is restricted to initial rates, that is, the rate in the absence of products, and this rate is determined by some method of extrapolating back to the time of mixing of enzyme and reactants. Next, in the many systems involving two or more reactants, it is customary to provide a large excess of all reactants but one. In this way the rate is made to appear proportional to the concentration of the single limiting substrate.

Actually in the hydrolysis of sucrose in solutions of ordinary concentration catalyzed by invertase (sucrase) the rate is controlled by

$$\text{Sucrose} + H_2O \xrightarrow{\text{invertase}} \text{glucose} + \text{fructose}$$

the concentration of sucrose, and the relatively small changes in the water concentration concurrent with changing sucrose concentration normally have no detectable effect. However, at high sucrose concentrations, anomalies in the application of the simplified equations occur, apparently because the effective concentration of the water becomes important. When a gas is taken up during a reaction, this dependence of rate on all reactants is even more manifest. In such cases the partial pressure controls the solubility and thus the rate of reaction.

Regardless of any simplifying assumptions made for mathematical purposes, the rate of reaction depends upon the concentration of enzyme. In some characteristic range of concentration, there is direct proportionality. Above this range the relationship changes until finally further increases in enzyme concentration have no effect on the rate. In this respect an enzyme behaves like an ordinary reactant.

Assuming that the concentration of only one reactant is limiting, the initial reaction velocity, v, is given by

$$v = \frac{k_3[E][S]}{K_m + [S]}$$

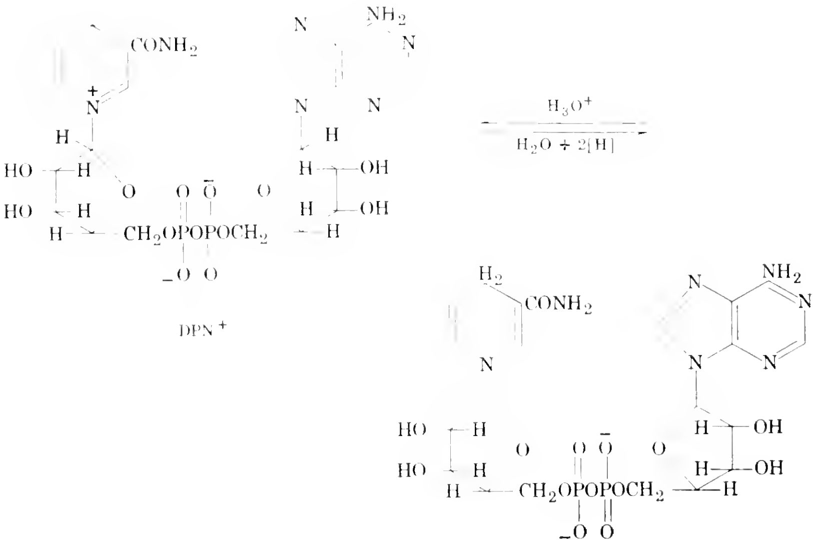
where [E] and [S] are the concentrations of enzyme and substrate, respectively, and k_3 and K_m are constants characteristic of the enzyme. Thus, in enzyme-catalyzed reactions the initial velocity is proportional to enzyme concentration and related to substrate concentration. Although the constants can be evaluated and are characteristic of the enzyme-substrate system, their major value lies in work with enzyme inhibitors (see page 145).

Cofactors

Many enzymes act on their substrates only in the presence of additional materials called cofactors. These substances are readily separated from the enzymes themselves and are usually not isolated with the enzymes. Thus cofactors are distinguished from the firmly bound prosthetic groups. The cofactors are readily dialyzable, small molecules that are relatively heat stable. At least in some cases, they are known to form complexes with their respective enzymes during the course of enzymatic activity. These complexes are relatively unstable, with equilibrium points permitting easy removal of the cofactors.

The cofactors may be divided into two groups. When the cofactor itself undergoes a definite chemical reaction, it is usually called a coenzyme. Such changes would classify the coenzymes with substrates, and sometimes there may be no real distinction. However, the chemical roles of the coenzymes were unknown when they were first discovered, so the term has been retained. Besides, the number of different coenzymes is much more limited than the number of substrates, and a single coenzyme functions with many different enzymes and substrates. Finally, though the structure of a coenzyme is changed by an enzymatic reaction, in a functioning cell a subsequent enzymatic process restores the coenzyme to its original form. Substrates usually undergo successive transformations, seldom being re-formed except in certain cyclic processes. Thus the use of the special term *coenzyme* seems justified.

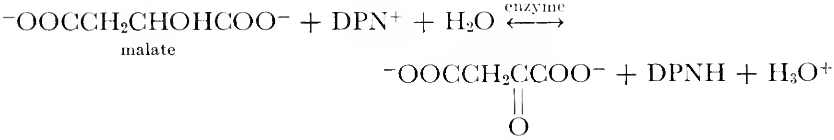
It turns out that several of the water-soluble vitamins are important structural components of certain coenzymes. Since fifteen or more coenzymes are now known and most are rather complex in structure, only two examples will be presented here. These two have been chosen from among coenzymes involved in quite diverse roles, including the transfers of phosphate, hydrogen and electrons, and acyl groups; the amination, deamination, and decarboxylation of amino acids; the decarboxylation of keto acids; and the isomerization of carbohydrate derivatives. One coenzyme, variously called diphosphopyridine nucleotide, DPN^+, coenzyme I, cozymase, or codehydrogenase I, participates with specific enzymes in a number of oxidation-reduction systems of great metabolic importance. The coenzyme itself is oxidized or reduced according to the following scheme:



DPN⁺

DPNH

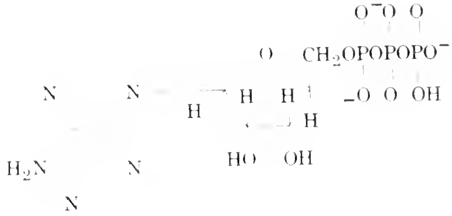
The reduced form at the right has gained two electrons and one proton by this reaction and is called reduced diphosphopyridine nucleotide or DPNH. Note that the upper left group corresponds to nicotinamide, one of the B vitamins. The purine is adenine, the sugar D-ribose, and the combination with phosphate is adenylic acid (see page 122). [H] represents a suitable source of hydrogen atoms. The above reaction occurs in connection with a number of enzymatic processes, for example:



$$\underset{\text{malate}}{^{-}OOCCH_2CHOHCOO^{-}} + DPN^{+} + H_2O \xleftrightarrow{\text{enzyme}} \underset{\text{oxalacetate}}{^{-}OOCCH_2\underset{\underset{\displaystyle O}{\|}}{C}COO^{-}} + DPNH + H_3O^{+}$$

The enzyme specific for this reaction is called malic dehydrogenase, and the double-pointed arrow signifies reversibility. The reaction goes in either direction, depending upon the concentrations of the materials present. As is now customary, malate and oxalacetate are written as the ions rather than as the free acids malic and oxalacetic, since these ions are the forms natural to the cells employing this reaction.

Another coenzyme, called adenosine triphosphate or ATP, participates in the transfer of phosphate groups. As shown in the following structure, this particular substance is closely related to adenylic acid and to one half of DPN+. This coenzyme functions by transfer of the terminal phosphate to a substrate molecule in the presence of any one of several enzymes. This loss of a phosphate group yields



ATP

adenosine diphosphate (ADP), which under certain conditions can transfer one of its phosphates and turn into adenylic acid (adenosine monophosphate). The first biochemical step in the metabolism of glucose illustrates the role of ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow[\text{Mg}^{++}]{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

The specific enzyme requires a second cofactor, magnesium ion filling this role in nature. Other metabolic steps add phosphate to the ADP, reforming the ATP utilized above. Thus a coenzyme consumed at one stage and transformed into another substance is ultimately restored by some subsequent reaction.

The second group of cofactors includes materials, mainly inorganic ions like the magnesium above, called activators. As the name suggests, the activators are required for or markedly accelerate enzyme action. They probably play roles in enzyme-substrate complexes, perhaps serving as links between enzyme and substrate. Activators also participate in systems involving coenzymes, so they may mediate interactions between enzyme, coenzyme, and substrates. Activators are not always completely specific, and it is usually possible to replace the ion functioning in the cell with a chemically related ion that works nearly as well. In fact the selection of the natural activator from among the list of ions that work can be very difficult when two or more of the possibilities exist in the cell. Magnesium and manganous ions are natural activators for several enzyme systems. Chloride, potassium, molybdenum, zinc, and calcium ions probably serve in certain cases, and other ions undoubtedly do so in still other systems.

Temperature

Changes in temperature characteristically change the rates of all reactions, including those involving enzymes. As the temperature rises, reaction rates increase. The exceptions to this statement are more apparent than real and involve phase changes or the occurrence of competing reactions and the like.

Reactions of isolated enzyme systems normally accelerate as the temperature rises through the physiological range. However, eventually a characteristic optimum temperature is reached and further increases cause a decrease in rate. Although harder to establish, this same phenomenon occurs for enzymic reactions in cells. In most cases these maximum rates lie at temperatures where the participating enzymes begin to undergo appreciable denaturation. From this point on, then, the higher the temperature, the more enzyme is denaturated and the lower the overall catalytic rate, since this rate is directly proportional to enzyme concentration.

Mathematical equations describing the relationship between temperature and reaction rate are available but are omitted here for two reasons. First, the mathematics itself is beyond the scope of this book. Second, the equations are largely limited to isolated and purified enzyme systems and to reactions in the absence of complexities like denaturation.

In general, these expressions depend upon the idea that molecules must be activated beyond some definite energy level before reaction can occur. As shown on page 22 a rise in temperature increases the fraction of molecules sufficiently activated to undergo reaction, and the rate increases. Enzymes themselves are believed to accelerate reactions by somehow lowering the energy level required before the substrate can react. Presumably this reduction in the threshold activation energy is a property of the enzyme-substrate complex, including all necessary cofactors. Possibly chemical bonds of the substrate are strained or otherwise destabilized in the complex, making them susceptible to reaction at lower energy levels and lower temperatures. Or perhaps the enzyme molecule serves as an energy source, trapping and transferring sufficient energy to the substrate to activate it for reaction.

pH

Changes in the pH of enzyme systems alter the rates of the reactions by exerting any one or any combination of three different influences. The overall effect leads to the occurrence of an optimum pH, a range of maximum rate. A shift of pH away from the optimum in either direction produces a reduction in rate. The effects due to changes in pH can be explained in particular cases but are usually too complex for complete description. Hence, it is more appropriate to consider generally the factors that can play a part and definitely do in particular instances.

In the first place, changes in pH modify the structure of the enzyme by adding protons to or removing them from the polar groups of the enzyme molecule. A change in a polar group affects the entire interdependent electronic structure of the molecule and alters the catalytic activity. If the group titrated is not directly involved in the reaction, the change in rate will be relatively small. However, when the group titrated is fundamental to the reaction, perhaps as the site of the linkage of substrate to enzyme, the effect of change in pH is marked. It is believed that there is a preferred form for any polar group directly involved. For example, when a histidine unit is the site of activity, pH changes around neutrality should produce dramatic changes in enzymatic activity, since the imidazole group of histidine is half charged, half uncharged at about pH 6.

A still more general effect of pH on enzyme activity occurs at more extreme values of pH. Most enzymes are inherently unstable in strongly acidic or alkaline solutions and denature. Since reaction rate depends on enzyme concentration, denaturation of enzyme automatically affects the rate. At the point where the effect of denaturation overcomes any existing compensating gains in rate arising from substrate effects and the like, the overall reaction begins to fall.

There are a few exceptional cases of enzyme stability at extremes of pH. Pepsin, for instance, has an optimum activity in the range of 1.5 to 2.0 and is rather stable in this region at ordinary temperatures. On the other hand, pepsin is unusually sensitive to alkali and is almost completely inactivated at pH 8.0. It may be argued that pepsin is fitted for activity in the very acid environment of the stomach and that evolution has selected a molecular type suitable for the situation.

In addition to changes of the enzyme, a shift in pH may also modify the substrate. A large majority of the substrates for enzymes possess one or more ionizable groups. Any changes in the ionic states of these groups affect not only the basic stability of the substrate molecule but also the interaction of substrate and enzyme to form the reactive complex. In the known cases only one form of substrate shows much tendency to combine with the enzyme and undergo reaction. Thus the equation of page 138 cannot be used for rate comparisons at different pH values because no account is taken of changes in the concentration of the active form of the substrate. Knowledge of the appropriate equilibrium constants might be incorporated into this equation, and this aspect of pH effects taken into account. Usually such a modified equation is not employed because it does not recognize the other effects of pH and because the identification and measurement of the proper equilibrium constants are difficult.

The third type of pH effect arises from participation of hydrogen ions in enzymatic reactions. In the reaction of page 140, as in a great many others, hydrogen ions are formed. In this reversible system they disappear, of course, when the reaction proceeds from right to left. As either a reactant or a product, the concentration of the hydrogen ion affects the extent of reaction through application of the law of mass action. Using another system as an example,

$$\underset{\text{lactate}}{CH_3CHOHCOO^-} + DPN^+ + H_2O \xrightleftharpoons[\text{dehydrogenase}]{\text{lactic}} \underset{\text{pyruvate}}{CH_3COCOO^-} + DPNH + H_3O^+$$

the equilibrium constant becomes

$$K = \frac{[CH_3COCOO^-][DPNH][H_3O^+]}{[CH_3CHOHCOO^-][DPN^+][H_2O]}$$

As usual the concentration of water is relatively great and little altered by the reaction, so this factor is incorporated into the constant. There is also a relationship between the equilibrium constant K and the velocity constants. When k_1 is the velocity (rate) constant for the reaction from left to right and k_2 the constant for the reverse reaction,

$$K = \frac{k_1}{k_2}$$

The actual rates of the reactions are proportional to these rate constants and are given by

$$R_1 = k_1[CH_3CHOHCOO^-][DPN^+][H_2O]$$
$$R_2 = k_2[CH_3COCOO^-][DPNH][H_3O^+]$$

with the concentration of water usually ignored. The equations show the interdependence of the rate of either reaction on the pH. The oxidation of lactate ion is initially independent of pH in the absence of pyruvate and DPNH as far as this kind of consideration goes because there can be no reverse reaction without both pyruvate and DPNH. However, as soon as a trace of these materials forms, the reverse reaction begins. The overall reaction rate at any instant is the difference between the absolute rates of the two individual reactions, and the reverse step is shown to depend upon [H_3O^+]. Starting with pyruvate, the pH plays an initial and continuing role and becomes a key factor in the rates of enzyme systems like these. Thus a shift in pH changes not only the extent of reaction as predicted from the equilibrium constant but the net rate of reaction as well.

Inhibitors

Any substance whose presence reduces the rate of an enzyme reaction is called an inhibitor. The use of the word *substance* excludes the ordinary physical factors like heat; however, it does include a number of protein denaturants that reduce reaction rates by irreversibly destroying the enzymes. These general reagents include strong acids or alkalis, strong oxidizing agents, trichloroacetic acid, and several heavy-metal ions. Their mode of action is quite clearly a simple matter of reduction in the concentration of active enzyme.

Another type of inhibitor is much more specific, affecting only certain systems. These compounds are believed to form reversible complexes with the specific enzymes but complexes which do not react to form products. Assuming that enzyme E and substrate S form a complex ES which decomposes into enzyme plus products P, the following equilibrium may be written:

$$\mathrm{E} + \mathrm{S} \leftrightharpoons \mathrm{ES} \leftrightharpoons \mathrm{E} + \mathrm{P}$$

In the presence of one of these inhibitors I,

$$\mathrm{E} + \mathrm{I} \leftrightharpoons \mathrm{EI}$$

without a subsequent step leading to any products other than enzyme and inhibitor. Thus the inhibitor competes with the substrate for the enzyme and decreases the net amount of enzyme available for reaction with the substrate. According to

$$v = \frac{k_3[\mathrm{E}][\mathrm{S}]}{K_m + [\mathrm{S}]}$$

from page 138, the reaction rate automatically must be decreased. It is also apparent from this equation that when [S] is smaller than K_m an increase in [S] increases the rate. Hence the addition of substrate tends to overcome the effect of the inhibitor. This competition between substrate and inhibitor occurs when enzyme and inhibitor interact reversibly to form a complex to which the substrate does not attach. Inhibitors of this group are termed competitive.

Non-competitive inhibition occurs when addition of substrate does not reverse the effect of the inhibitor even though the reaction of inhibitor and enzyme is reversible. There are at least two mechanisms by which this situation can arise. First,

$$\begin{aligned} E + I &\leftrightarrows EI \\ EI + S &\leftrightarrows SEI \end{aligned}$$

Here the inhibitor combines with the enzyme at some point other than the enzymatically active site, allowing the substrate to take its normal position. However, the inhibitor exerts at a distance enough influence to block the formation of products. The addition of more substrate has little effect on the rate, and there is no competition between enzyme and inhibitor.

Another type of non-competitive inhibition occurs when the inhibitor reacts with the enzyme-substrate complex

$$ES + I \leftrightarrows ESI$$

to prevent the formation of products. Again the increase in substrate concentration has little effect on the catalytic rate in the presence of the inhibitor. Finally, inhibition can occur when

$$S + I \leftrightarrows SI$$

and this equilibrium also leads to the non-competitive type.

Much work on enzyme inhibition has been carried on merely as a study of the nature of the phenomenon. But in the process something has been learned about the nature of the reactive sites of certain enzymes. In addition, there is much practical interest in such studies because many of the antibiotics are thought to be enzyme inhibitors. This medically important class blocks key enzyme reactions in pathogenic organisms when administered in doses too small to be harmful to the animal concerned.

A few effective inhibitors for enzymes have turned out to be proteins themselves. A protein from soybean prevents the proteolytic action of trypsin and is thus called trypsin inhibitor. Although trypsin normally catalyzes the hydrolysis of proteins, it does not attack this one, probably because the inhibitor possesses no exposed peptide bonds of the types susceptible to trypsin. The inhibitor and enzyme do, however, form a stable complex preventing the enzyme from combining with molecules of substrate protein.

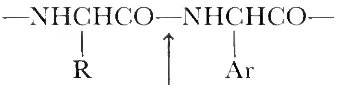
TYPICAL ENZYMES

The number of known enzymes is very large and still increasing rapidly. For both reasons a complete listing of enzymes is impractical. However, a few of the more widely studied representatives of the six major groups of the classification system have been chosen as typical examples, beginning with the various types of hydrolytic enzymes. Many of the enzymes mentioned below will be discussed again in later chapters.

Proteases

These enzymes catalyze the partial hydrolysis of proteins, peptones, and peptides; in fact, almost anything containing the required specific peptide bond. However, the rates of action are generally much higher for proteins than for small peptides. The proteases, also called proteinases or proteolytic enzymes, probably occur in almost all cells. They are especially abundant in those plant and animal tissues which hydrolyze proteins extensively. Each protease is characterized by specificity toward particular peptide bonds. Most are rather specific, hydrolyzing only a few types of peptide linkages.

Pepsin and rennin are gastric enzymes functioning at the relatively low pH of the stomach. Rennin is found in the stomachs of young animals like the calf and the lamb, where it clots milk and starts the hydrolysis of milk proteins. As the animal grows older the rennin diminishes and is replaced by pepsin. This latter enzyme is secreted in the form of the precursor pepsinogen, which is converted to active pepsin in the presence of acid. Pepsin plays a role in digestion in birds and mammals by specifically attacking peptide bonds of the type



$$-\mathrm{NH}\underset{\substack{|\\ \mathrm{R}}}{\mathrm{C}}\mathrm{HCO}\underset{\uparrow}{-}\mathrm{NH}\underset{\substack{|\\ \mathrm{Ar}}}{\mathrm{C}}\mathrm{HCO}-$$

where R signifies the side chain of any of the usual amino acids but Ar represents only aromatic groups. Bonds involving the carboxyl groups of aromatic amino acids are not attacked unless attached to other aromatic amino acids.

Trypsin and chymotrypsin are secreted by the pancreas in the form of inactive precursors which are both activated catalytically by trypsin itself. These and related enzymes function best in neutral or slightly alkaline solutions. Trypsin acts on the peptide bonds involving the carboxyl groups of lysine or arginine. Chymotrypsin splits the peptide bonds at the carboxyl end of the aromatic amino acids. Both have hydrolytic activity for methyl and ethyl esters of peptides, although the biological importance of this action is unsettled. Insectivorous plants have related enzymes.

Papain from papaya, ficin from fig, and bromelin from pineapple are typical of the papainase group of proteolytic enzymes from plants. Many require free —SH groups for activity and are inactivated by heavy metals or oxidation. These enzymes are widely distributed in fruits, microorganisms, and seeds. Occasionally they are encountered in the digestion of gelatin, thus preventing the setting of gelatin desserts. The papainases have a wide range of specificity, attacking many different peptide bonds.

Most of the preceding proteases are exocellular enzymes and function after secretion by the cell. Another important group, though not well known, called cathepsins, act inside cells. These enzymes are thought to be inactive while the cells live. When a cell dies its pH drops, activating the cathepsins, which hydrolyze the cellular proteins as a part of the change resulting from death.

Esterases and Lipases

These enzymes catalyze the hydrolysis of esters and lipides into the individual acids and alcohols. They are very widely distributed in both plants and animals, but much less is known of most of them than of the proteases.

Pectase or pectinase attacks pectin, and tannase attacks the complex tannins. Both enzymes are known only from plants. Choline esterase from animals is important in the conduction of nerve impulses hydrolyzing acetylcholine to choline and acetic acid.

$$CH_3COOCH_2CH_2N^+(CH_3)_3 + H_2O \xrightarrow[\text{esterase}]{\text{choline}} CH_3COOH + HOCH_2CH_2N^+(CH_3)_3$$

The enzyme is biologically so important to higher animals that some of its inhibitors are chemical warfare agents called the nerve gases.

Lipases act on the lipides of both plants and animals. These enzymes yield the separate components of both fats and oils for further metabolic transformations. Pancreatic lipase (steapsin) is fairly typical, playing an important role in animal digestive tracts. It is activated by calcium ions. Since the substrates are ordinarily not water soluble, the enzymatic action is markedly accelerated by emulsifying agents which help disperse the non-polar substrate molecules.

The natural dispersing agents involved are called bile salts and are secreted by the liver.

Carbohydrases

This group includes those enzymes hydrolyzing the various glycosidic linkages between sugar units or between sugar and alcohol. The bonds affected are of the acetal type and are split during digestive processes. Carbohydrases are universally distributed and usually possess relatively sharp specificities. For example, some attack only acetals having the β configuration of a particular monosaccharide subunit in the substrate molecule.

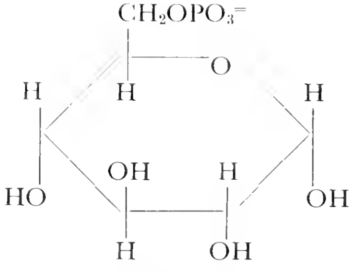
The amylases attack the 1,4-α-glucosidic linkages of starch and glycogen. α-Amylase splits bonds near the middle of the amylose or amylopectin molecules, yielding mainly large fragments, dextrins, but liquefying starch gels in the process. β-Amylase hydrolyzes the same bonds but works from the ends of the chains inward, breaking off maltose segments one after another. This enzyme splits amylose completely but does not proceed past the branches in amylopectin. The 1,6-α-glucosidic linkages of these branches prevent action past that point in the amylopectin residue. Further digestion requires the activity of another enzyme, 1,6-α-glucosidase, in removing the branches.

Cellulase hydrolyzes the 1,4-β-glucoside bonds of cellulose to form a disaccharide, cellobiose, which is then split to glucose by cellobiase. These two enzymes, cellulase and cellobiase, are important in the digestion of cellulose by micro-organisms. Cellulase is not synthesized by animals which, therefore, cannot utilize cellulose directly. Ruminants and horses, for example, possess bacterial flora in the rumen and intestine, respectively, capable of degrading cellulose into fragments digestible by these animals.

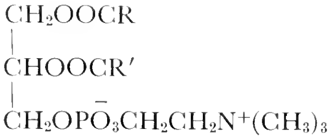
Maltase and emulsin are representative of the enzymes specific for small carbohydrates. Maltase (α-glucosidase) cleaves maltose and a variety of substances that are acetals of α-D-glucose, including α-methyl glucoside. Emulsin (β-glucosidase) requires acetals of the β configuration. In this case, sugars other than glucose exist in susceptible substrates.

Phosphatases

These enzymes hydrolyze either esters or anhydrides of phosphoric acid. The types of substrates include monoesters like glucose-6-phosphate, diesters of phosphoric acid like lecithins and nucleic acids, and anhydrides like acetyl phosphate, pyrophosphate, and adenosine triphosphate (structure on page 141).



glucose-6-phosphate



$$\begin{array}{l} CH_2OOCR \\ | \\ CHOOCR' \\ | \\ CH_2OP\bar{O}_3CH_2CH_2N^+(CH_3)_3 \end{array}$$

a lecithin

$CH_3COOPO_3^=$ acetyl phosphate

$H\bar{O}_3POPO_3^=$ pyrophosphate

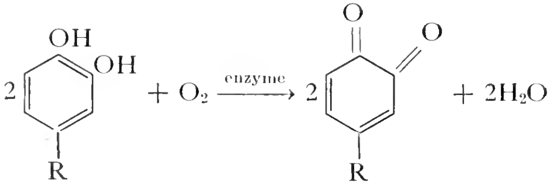
The phosphatases are important in the digestion of compounds of these types, in the maintenance of a sufficient supply of inorganic phosphate, and in the metabolic reactions of phosphorylated materials.

Amidases and Peptidases

These enzymes split amides and peptides of various kinds. They are widespread and act on natural amides and peptides but not ordinarily on proteins. The specificity may be sharp or slight, and they play important roles at the end of digestive processes yielding very small peptides, amino acids, and ammonia. As an example, urease is distributed among plants, animals, and microorganisms and hydrolyzes urea into ammonia and carbonic acid.

Oxidases

Among the oxidation-reduction enzymes the oxidases are especially important to all aerobic cells because certain of these enzymes provide for the use of molecular oxygen as an oxidizing agent. This arrangement is based on different enzymes in different cells. A number of plants contain oxidases catalyzing the oxidation of certain catechol derivatives according to:



The enzymes of this group are known by many names, including tyrosinase and catecholase. The product of the reaction, a quinone, is reduced by some other cellular material and then undergoes another reaction cycle, thus serving to shuttle protons and electrons to oxygen and forming water. Such a system operates in plants like apples, bananas, mushrooms, and potatoes. When cellular function is seriously impaired, the supply of suitable reducing agents may be interrupted. The quinone accumulates and spontaneously polymerizes to the colored materials characteristic of exposed slices of apple or potato. This same sort of process occurs during the formation of some black and brown pigments in insects, animals, and tumors.

The principal system for the utilization of oxygen by animal cells and by some microorganisms and plant tissues involves the cytochromes. These compounds are proteins possessing prosthetic groups that contain iron. A complex series of reactions transfers electrons from the ferrous ions of cytochromes to oxygen, and hydrogen ions disappear from the solution.

$$4\ \text{Cytochrome-Fe}^{++} + O_2 + 4H_3O^+ \xrightarrow[\text{oxidase}]{\text{cytochrome}} 4\ \text{cytochrome-Fe}^{+++} + 6H_2O$$

The ferric form is reduced by the last of a sequence of reactions going back to the DPNH formed in reactions like that of page 140. The enzyme cytochrome oxidase catalyzes the transfer of electrons from the last cytochrome of the series to oxygen. This enzyme may contain iron, although some workers believe that copper is present instead. At points along the reaction chain chemical energy is available for use in the physiological processes of the cell. The cytochrome system will be referred to again.

Some oxidases lead to hydrogen peroxide instead of water, but the biological importance of these systems is not known. The hydrogen peroxide formed is toxic and must be decomposed, probably by catalase or peroxidases.

Dehydrogenases

This group of oxidation-reduction enzymes participates in the transfer of hydrogen from one substance to another. Both donor and acceptor can be and usually are quite specific. The number of known dehydrogenases is rather large, and many of them have been intensively studied. The reactions catalyzed by lactic and malic dehydrogenases have already been mentioned. Succinic dehydrogenase is another widespread example and acts upon succinate.

$$\underset{\text{succinate}}{{}^-OOCCH_2CH_2COO^-} \xrightarrow{\text{enzyme}} \underset{\text{fumarate}}{{}^-OOCCH{=}CHCOO^-} + 2H$$

Succinic dehydrogenase occurs in insoluble form in an enzymatically active particle. It appears to be a flavoprotein complexed with cytochrome *b*. The dehydrogenases are essential to the functioning of all metabolizing cells.

Transphosphorylases

This group of enzymes fits into the important and universal class of transferring enzymes. Transphosphorylases catalyze exchanges of phosphate groups from one substrate or coenzyme to another. Most are rather specific with definite requirements for substrate, coenzyme, and activator. The reactions are fundamental to the energy transfers of cells and participate in the conversion of the chemical energy of substrates into energy for growth, repair, body heat, and mechanical activity. Additional information on these processes appears in Chapter 7.

Hexokinase, also called glucokinase, has already been mentioned. It transforms glucose and ATP into glucose-6-phosphate and ADP when activated by magnesium ions, thus beginning the utilization of glucose. Corresponding enzymes phosphorylate fructose, galactose, pyruvate, arginine, and other related compounds and their derivatives.

Transglycosidases

These enzymes are important in carbohydrate syntheses and transfer monosaccharide units from one substrate to another. These reactions are probably all reversible, serving either the formation or the breakdown of carbohydrates, depending on the needs of the cell. For example, phosphorylase catalyzes the formation of reserve carbohydrates when activated by magnesium.

$$\text{Glucose-1-phosphate} + (\text{glucose})_n \leftrightarrows (\text{glucose})_{n+1} + \text{HPO}_4^{=}$$

The invertases fall in this group, converting sucrose into glucose and fructose by way of larger carbohydrates. Enzymes of this group also form sucrose and maltose.

$$\text{Glucose-1-phosphate} + \text{fructose} \xrightleftharpoons[\text{phosphorylase}]{\text{sucrose}} \text{sucrose} + \text{HPO}_4^{=}$$

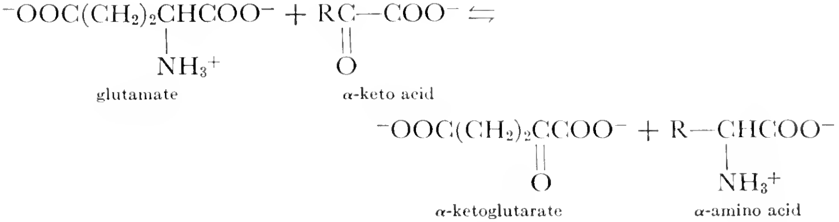
Still others transfer ribose to pyrimidines, purines, and nicotinamide to form the nucleosides.

Transpeptidases

Transpeptidases transfer or exchange amide, hydroxylamine, or amino acid groups. Although these activities are fairly well known, the extent of their biological occurrence is still in doubt.

Transaminases

The reactions catalyzed require a coenzyme derived from the B_6 vitamins shown on page 340. The general process may be written thus:

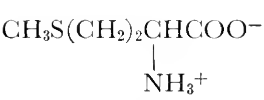


$$\underset{\text{glutamate}}{{}^{-}OOC(CH_2)_2\underset{\underset{NH_3^+}{|}}{C}HCOO^-} + \underset{\alpha\text{-keto acid}}{R\underset{\underset{O}{\|}}{C}—COO^-} \rightleftharpoons \underset{\alpha\text{-ketoglutarate}}{{}^{-}OOC(CH_2)_2\underset{\underset{O}{\|}}{C}COO^-} + \underset{\alpha\text{-amino acid}}{R—\underset{\underset{NH_3^+}{|}}{C}HCOO^-}$$

Alanine, aspartic acid, and perhaps other amino acids are formed primarily according to this reaction, which thus becomes important for the subsequent synthesis of proteins.

Transmethylases

A series of enzymatic reactions transfers methyl groups from one compound to another, leading to the formation of choline, methionine, betaine, creatine, serine, and a number of related compounds, all of metabolic importance.

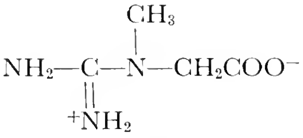


$$CH_3S(CH_2)_2\underset{\underset{NH_3^+}{|}}{C}HCOO^-$$

methionine

$$(CH_3)_3\overset{+}{N}CH_2COO^-$$

betaine



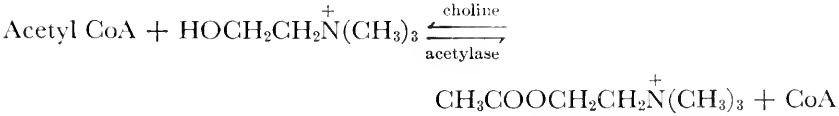
$$NH_2—\underset{\underset{{}^{+}NH_2}{\|}}{C}—\overset{\overset{CH_3}{|}}{N}—CH_2COO^-$$

creatine

There are still many uncertainties in the overall sequence of reactions, but much progress is being made.

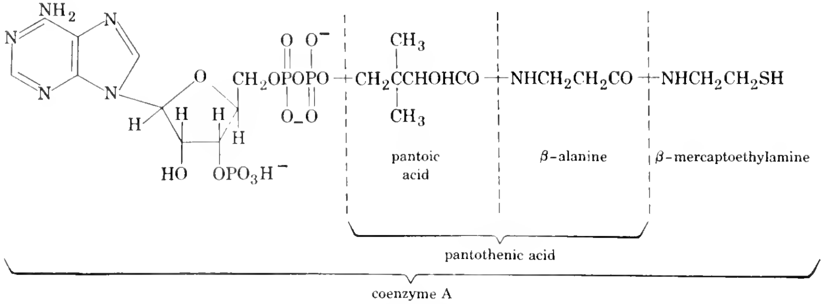
Transacylases

Transacylases catalyze transfers of acyl groups, for example, in the synthesis of acetylcholine by means of an acyl derivative of coenzyme A (CoA).



$$\text{Acetyl CoA} + HOCH_2CH_2\overset{+}{N}(CH_3)_3 \underset{\text{acetylase}}{\overset{\text{choline}}{\rightleftharpoons}} CH_3COOCH_2CH_2\overset{+}{N}(CH_3)_3 + \text{CoA}$$

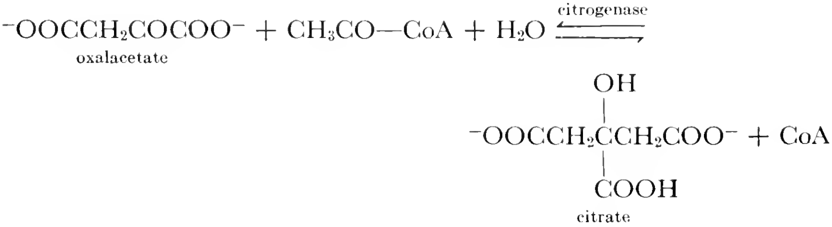
Coenzyme A participates in several reactions in the metabolism of carbohydrates and lipides. Its structure is as follows:



where the dotted lines separate the overall structure into the components named. The left-hand segment is a phosphate derivative of adenosine diphosphate, ADP. Coenzyme A is acylated on the thiol group as in

$$CH_3COCOO^- + DPN^+ + CoA \xrightarrow[\text{other cofactors (see page 171)}]{\text{pyruvic dehydrogenase}} CH_3CO{-}CoA + CO_2 + DPNH$$

Thereafter, the acetyl group may be replaced by other acyl groups or transferred to other substrates to form new types of materials, as in the following:

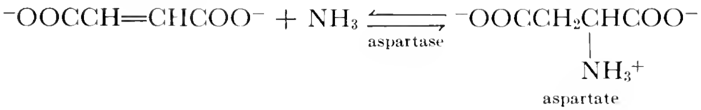


$$\underset{\text{oxalacetate}}{^-OOCCH_2COCOO^-} + CH_3CO{-}CoA + H_2O \overset{\text{citrogenase}}{\rightleftharpoons} \underset{\text{citrate}}{^-OOCCH_2\overset{\overset{\displaystyle OH}{|}}{\underset{\underset{\displaystyle COOH}{|}}{C}}CH_2COO^-} + CoA$$

Addition Enzymes

This group might include all enzymes catalyzing any condensation or combination of reactants. However, it is considered here to cover only additions of water or ammonia to double bonds between carbon atoms. Typical examples are:

$$\underset{\text{fumarate}}{^-OOCCH{=}CHCOO^-} + H_2O \underset{\text{fumarase}}{\rightleftharpoons} \underset{\text{malate}}{^-OOCCH_2CHOHCOO^-}$$



$$^-OOCCH{=}CHCOO^- + NH_3 \underset{\text{aspartase}}{\rightleftharpoons} \underset{\text{aspartate}}{^-OOCCH_2\underset{\underset{\displaystyle NH_3^+}{|}}{C}HCOO^-}$$

Isomerizing Enzymes

These enzymes fall into two categories, those inducing changes in the configurations of carbon atoms and those shifting groups from one carbon atom of the substrate to another. The interconversion of the stereoisomers of alanine and the mutarotation of glucose are examples of the first type.

$$\textsc{d}\text{-Alanine} \underset{\text{racemase}}{\overset{\text{alanine}}{\rightleftharpoons}} \textsc{l}\text{-alanine}$$

$$\alpha\text{-}\textsc{d}\text{-Glucose} \overset{\text{mutarotase}}{\rightleftharpoons} \beta\text{-}\textsc{d}\text{-glucose}$$

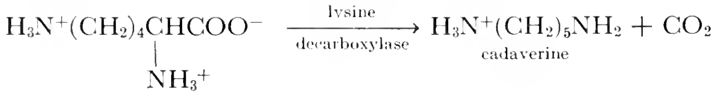
Among the second group of isomerizing enzymes is found the reaction

$$\underset{\substack{\text{dihydroxyacetone}\\ \text{phosphate}}}{\text{HOCH}_2\text{COCH}_2\text{OPO}_3^{=}} \underset{\text{triosephosphate isomerase}}{\rightleftharpoons} \underset{\substack{\text{3-phosphoglycer-}\\ \text{aldehyde}}}{\text{OHCCHOHCH}_2\text{OPO}_3^{=}}$$

Carboxylation Enzymes

These enzymes include many systems in which carbon dioxide is a reactant or a product. The reactions involve carboxyl groups and lead to either the formation or the removal of such groups.

$$\text{CH}_3\text{COCOO}^- + \text{H}_3\text{O}^+ \underset{\text{carboxylase}}{\overset{\text{pyruvic}}{\rightleftharpoons}} \text{CH}_3\text{CHO} + \text{CO}_2 + \text{H}_2\text{O}$$



$$\underset{\substack{|\\ \text{NH}_3^+}}{\text{H}_3\text{N}^+(\text{CH}_2)_4\text{CHCOO}^-} \xrightarrow[\text{decarboxylase}]{\text{lysine}} \underset{\text{cadaverine}}{\text{H}_3\text{N}^+(\text{CH}_2)_5\text{NH}_2} + \text{CO}_2$$

The second reaction requires pyridoxal phosphate as the coenzyme. A number of these carboxylation-decarboxylation systems combine to form the carbon dioxide evolved during respiration. One or more reversible steps utilize the carbon dioxide taken up during photosynthesis.

Practical Utilization of Enzymes

Enzymes were used as catalysts for various reactions for hundreds of years before they were recognized as definite chemical substances. The description of the agents (ferments) which produced fermentations precedes our knowledge of the existence of enzymes in living cells. Enzymatic processes such as bread-making, brewing, alcohol manufacture, and wine-making have been known from antiquity.

One of the older applications of enzymes still employed is the use of rennin or rennet to make cheese with the enzyme coagulating the casein of milk. Commercial rennet is prepared from the fourth or true stomach of the calf. Preservatives such as boric acid, benzoic acid, or large amounts of sodium chloride are sometimes added to prevent decomposition of the enzyme preparations by bacteria. Most of the millions of pounds of cheese produced annually in the United States is made with the aid of rennet.

The processing of fruit juices has grown from a very small industry 20 years ago to a relatively large one. This growth has been due in part to the ability of producers to clarify juices with the aid of enzymes. A mixture of pectic enzymes is added to the juice to hydrolyze the pectic substances causing turbidity.

All woven fabrics contain starch or other sizing applied to the warp threads to strengthen the yarn before weaving. If these fabrics are later bleached, printed, or dyed, this sizing must be removed, commonly by hydrolysis with amylases. The enzyme methods of desizing are more satisfactory than those employing acid or alkali because these latter agents attack cellulose and weaken the fabric. Several enzyme products for desizing cloth are manufactured in the United States from bacteria, fungi, malt, or other biological materials.

In the manufacture of leather, proteolytic enzymes prepared from such materials as the pancreas or *Bacillus mesentericus* are used to hydrolyze the proteins of the hair follicles, thus freeing the hair so that it may be readily scraped from the hides. Enzymes can be used in dry cleaning for the removal of stains due to glue, gelatin, or starch. Amylases are employed to prepare a partially hydrolyzed starch as surface coating for paper. Pepsin is used to digest gelatin in the process of recovering silver from photographic film.

Processes that employ microorganisms for the production of specific chemical substances are, in effect, applications of enzymology. Such processes produce lactic, acetic, citric, gluconic, and gallic acids, L-sorbose, acetone, butanol, ethyl alcohol, and many other products.

Digestive disturbances due to an insufficiency of enzymes have been treated for many years by supplying the lacking enzymes. Pepsin, papain, bromelin, and amylases aid digestion in the mouth and stomach. Pancreatic enzymes can be supplied by encapsulating them in material that is not dissolved in the stomach but is soluble in the juices of the duodenum. The enzymes are not released, therefore, until they arrive at the site of action.

Sloughing wounds and abnormal conditions such as bed sores, furuncles, ringworm, and other suppurative skin diseases have been alleviated by means of proteolytic enzymes from the pig pancreas.

These enzymes apparently destroy proteolytic systems which prevent the healing of such wounds.

A sterile papain hydrolyzate of meat placed in a solution of salt, glucose, and vitamins has been used in the treatment of chronic starvation in India. Similar preparations of protein hydrolyzates are now becoming increasingly important in feeding malnourished patients in certain types of postoperative cases and in diseases of the digestive tract.

Mixtures of amylases and maltase, called diastases, are used in analytical chemistry for starch determinations. The method employed measures the increase in reducing sugars resulting from the enzymic hydrolysis of starch. This method is more specific than one that measures the increase of reducing sugars after hydrolysis by acid.

Uricase is used in a method for the determination of uric acid in blood, and urease is employed for the determination of urea in blood and urine. The ammonia formed by urease is determined either by titration or by the color formed with Nessler's solution.

Sucrose and raffinose are determined in mixtures of sugars and in sugar products by polarimetry before and after treating the solutions with sucrase and melibiase. The activity of phosphatase present in milk is used as a measure for the efficiency of pasteurization. The inactivation temperature and time needed to destroy phosphatase activity are such that pathogenic bacteria in milk are killed before the enzyme is completely inactivated.

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Energy transfers and biological oxidations

7

All life depends upon the ability to utilize energy from the environment for the physiological processes of the living system. The useful energy is available in three general forms. Light is used directly by the photosynthetic plants and microorganisms employing the mechanism outlined on page 213. Chemical energy is utilized in all species by the metabolic reactions of the sections beginning on page 166. Heat serves indirect roles in providing for the liquid internal phases of cells and suitable external environments for species living in water and soil. In addition, the temperature must be high enough to allow a suitably rapid metabolic rate but low enough to avoid denaturation of the proteins and other sensitive elements of the cells.

All cells require heat from the environment, and all probably supplement this heat by additional heat energy derived from the reactions carried out. Furthermore, all cells also require environmental energy in one of the other two forms. The photosynthetic plants, for example, convert light energy into stored chemical energy and are thus able to utilize it for the activities of life. Animals and the other species unable to convert light to chemical energy must secure the latter directly from the environment. The different living forms are characterized by the kinds of compounds capable of supplying the required chemical energy, some needing a variety of substances for special purposes, some subsisting on a very few.

The rate at which an organism processes the energy it receives influences the rate of growth, ultimate size, mechanical activity, rate of reproduction, and population size. Those species transferring energy most efficiently and rapidly tend to predominate in any ecological situation. At the same time they produce the greatest changes in the environment itself by markedly decreasing the available energy (at least the chemical forms) and releasing large quantities of waste products.

ENERGY TRANSFERS

As fast as energy is absorbed in the form of heat, light, or foodstuffs, it is made available to the various parts of the organism. Heat is transferred from molecule to molecule during the collisions resulting from their kinetic motions. Those molecules heated by the environment thus acquire kinetic energy which is shared with neighboring molecules after collisions. Animals utilize radiant energy, including visible light, in this way by absorbing it with a gain in the kinetic energy of molecules. Green plants also absorb light but are capable of converting much of this absorbed energy into chemical forms instead of heat. Special mechanisms (see page 206) are required for this process. Chemical energy is contained in substrates or foods which diffuse into or are ingested by the nonphotosynthetic species.

The chemical energy accumulated either from photosynthesis or directly from the environment is made available for the activities of the organism by means of the complex array of reactions called metabolism. The activities thus powered include maintenance of temperature, movement, growth, reproduction, repair, emission of light, generation of electricity, accumulation of foods or substrates, elimination of wastes. The metabolic reactions involved in all these functions are numerous, complex, and only partly known. Rapid progress is being made but complete understanding is not in sight. Moreover, different organisms possess different mechanisms in part, greatly complicating the task.

Energy Transfers in Metabolism

The chemical transformations of cells include many reactions which typically depend upon energy as either a reactant or a product. This chemical energy is commonly referred to as free energy, symbolized by the letter F. During a reaction at least part of the free energies of the reactants appears in the products. The difference is the free-energy change ΔF. Thus the reaction represented between A and B to give Y and Z may be written

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{Y} + \mathrm{Z} + \Delta F$$

For convenience the last sign is omitted and known values of ΔF may be inserted,

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{Y} + \mathrm{Z} \qquad \Delta F = 3{,}000 \text{ cal.}$$

with chemical energy expressed in calories like heat.

When the total free energies of the reactants exceed those of the products, the reaction is said to be spontaneous and will occur without an external supply of energy. This situation is arbitrarily represented with a negative sign in front of the value of ΔF. On the other hand, if the free energy of the products exceeds that of the reactants, energy must be supplied to keep the temperature up and the reaction going. In such cases as the hypothetical reaction above, the change in free energy is assigned a plus symbol or the sign is omitted. Practically all reactions fall into one group or the other with few possessing $\Delta F = 0$ and then only over a limited range of temperature. The presence of enzymes or other catalysts changes the rate of reaction but apparently does not change the value of ΔF.

A mathematical expression relates changes in free energy with heats of reaction. In practice the extra free energy required to make a reaction take place may be supplied as heat at the expense of either the system or the environment. In the former case, when energy is drawn from the reaction system without replacement from the environment, the temperature falls until the reaction slows down and finally ceases. In the latter case reaction proceeds until the reactants are consumed or equilibrium is reached.

Spontaneous reactions yield the excess free energy for direct utilization in subsequent reactions or for conversion to an equivalent amount of heat. This heat may produce a general increase in temperature, diffuse to the sites of reactions consuming free energy, or both.

Cells are distinctly limited in the temperatures they can withstand. Thus, if the free-energy change of a reaction were very large and were all transformed to heat, a fatal rise in temperature might occur. Likewise, the substitution of heat for a high free-energy requirement could lead to an excessive temperature. Therefore, reactions involving interconversions of heat and chemical energy can safely occur in a cell only if the free-energy changes are comparatively small. Many reactions in biological materials fit this condition.

Other processes produce relatively enormous changes in free energy as illustrated by the complete oxidation of glucose.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad \Delta F = -688{,}000 \text{ cal.}$$

Such a quantity of energy converted to heat would be immediately destructive. However, if this reaction can be carried out in a sufficient number of steps to reduce the energy yields at each to safe values, glucose would serve as a useful source of chemical energy. Evolution has provided the mechanism for such a reaction pathway.

Even though the oxidation of glucose by cells takes place by a number of steps, some of the ΔF values are still fairly large, over $-10{,}000$ cal. Moreover, reactions in the sequence require energies in this range, and use of heat intense enough for the purpose is undesirable. At such points in the system, reactions convert the free energy of one compound into that of another used later to drive a second reaction requiring a large input of free energy. These high-energy reactions are thus linked together not by heat but by the free energy of these special energy-transferring compounds. Such substances are called high-energy compounds from their metabolic function of transferring sizable quantities of free energy. These compounds are illustrated and discussed in the next section. Some of the metabolic reactions in which they participate appear in all the subsequent material devoted to metabolism.

High-energy Compounds

The biological substances usually placed in this category undergo hydrolysis with $\Delta F = -6{,}000$ to $-15{,}000$ cal. They are found in and participate in many of the reactions of all living cells. Indeed, they have been called a common denominator of life. During normal cell function, the energy inherent in these high-energy compounds is not set free by hydrolysis but is transferred by reaction to products which then undergo subsequent reactions along the metabolic pathway. In general terms the action of these versatile substances may be illustrated with

$$A + B \rightarrow C + D^*$$
$$D^* + L \rightarrow B + M$$

where the asterisk denotes a high-energy compound converted by the second reaction into its original form.

Adenosine triphosphate, ATP, is one of the most widely studied of the compounds whose role is primarily that of energy transfer. Its structure is shown on page 141. This compound is formed by a variety of metabolic steps, and the free energy thus stored is available for subsequent processes. The following specific example occurring during the utilization of glucose by common plants and animals shows how ATP participates in energy transfers.

$$\underset{\text{1,3-diphospho-D-glycerate}}{{}^{-}HO_3POCH_2CHOHCOOPO_3^{=}} + ADP \rightarrow \underset{\text{3-phospho-D-glycerate}}{{}^{-}HO_3POCH_2CHOHCOO^{-}} + ATP$$

ADP or adenosine diphosphate has a structure like that of ATP except that it contains one less phosphate group. For the present purpose the structure might be abbreviated to

$$\underset{\text{ADP}}{\text{Adenosine}—O\overset{-}{P}O_3P\overset{-}{O}_3H}$$

The reaction is catalyzed by a specific enzyme in the presence of magnesium ion and shifts a part of the free energy inherent in the 1,3-diphosphoglycerate to the resulting ATP when the phosphate group is transferred. The 3-phosphoglycerate undergoes the subsequent reactions of the pathway leading to compounds of lower free energy. However, the free energy stored in the ATP is used to drive various reactions employed for synthetic purposes in the cell or to modify foodstuffs for digestion and to get them into a form suitable for metabolism. Ramifications of a reaction already mentioned illustrate the biochemical effect of both this driving energy and certain subsequent reactions.

$$\text{Glucose} + \text{ATP} \xrightarrow[\text{glucokinase}]{Mg^{++}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} \xrightarrow[\text{reactions}]{\text{several}} \text{1,3-diphosphoglycerate, etc.}$$

$$\text{Glucose-6-phosphate} \longrightarrow \text{glucose-1-phosphate}$$

$$\text{Glucose-1-phosphate} + \text{fructose} \longrightarrow \text{sucrose} + HPO_4^{=}$$

$$\text{Glucose-1-phosphate} + \text{glucose} \longrightarrow (\text{glucose})_n + HPO_4^{=}$$

The last three reactions illustrate the synthetic role and result in the reserve carbohydrates made by plants and animals and used later in times of need. Enzymes and cofactors are required.

Coenzyme A shown on page 154 also plays a key part in energy transfer. A part of this compound resembles ADP and ATP and, like them, would show a large free energy on hydrolysis. However, during the energy transfers mediated by coenzyme A (CoA) the phosphate bonds are not directly involved. Rather the energy transfer is associated with transfers of acyl groups to and from the terminal thiol (—SH) group of the molecule. The importance of this group is emphasized by abbreviating the name and structure to CoA—SH and that of acetyl coenzyme A to $CH_3CO—S—CoA$. The free energy of the latter may be used as in

$$2CH_3CO{-}S{-}CoA \rightarrow \underset{\text{acetoacetyl coenzyme A}}{CH_3COCH_2CO{-}S{-}CoA} + CoA{-}SH$$

where the energy of one acetyl CoA has been used to make the new carbon-carbon bond in the acetoacetyl coenzyme A. This substance is a possible intermediate in the synthesis of fats by many cells. A more recent theory of fatty acid biosynthesis postulates a reduction of one acetyl coenzyme A molecule, then condensation of this product with a reactant from the other acetyl coenzyme A (see page 458) to yield β-hydroxybutyryl coenzyme A plus coenzyme A. This mechanism provides for reduction before condensation instead of the other way around, as above when acetoacetyl coenzyme A is formed. Acetyl coenzyme A is formed by the reversal of reactions like the above in the metabolism of fats and by a reaction of pyruvate mentioned earlier.

$$CH_3COCOO^- + HS{-}CoA + DPN^+ \rightarrow CH_3CO{-}S{-}CoA + CO_2 + DPNH$$

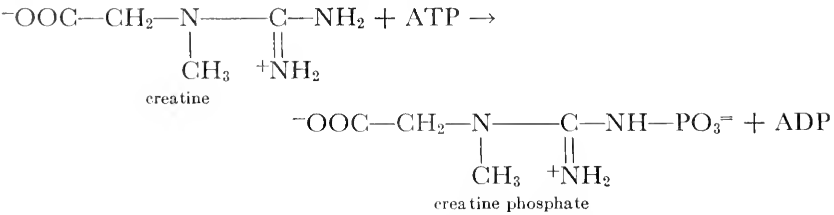
If not converted to fatty acids, the acetyl CoA may be converted to citrate as shown on pages 171 and 450, and from citrate the energy is recovered by later reactions.

Note that DPN^+ is reduced in the above reaction of pyruvate. This reduction utilizes part of the energy available when pyruvate is decarboxylated. Thereafter the DPNH formed (see page 140 for the structures) is reoxidized and the energy transferred to other substances. One of the most important steps is thought to be

$$DPNH + HOPO_3^= + ADP + H_3O^+ + FAD \rightarrow DPN^+ + ATP + FADH_2 + H_2O$$

where this obviously very complex process provides another source of ATP. Still other subsequent steps provide additional ATP from ADP and $HOPO_3^=$, although little is known about these steps. The structure of FAD (flavine adenine dinucleotide) appears on page 172.

The foregoing illustrates how chemical energy may be transferred and utilized for particular purposes. To some extent energy is stored in these special substances, ATP, $CH_3CO{-}S{-}CoA$, and DPNH. However, only limited quantities of these compounds accumulate in normal cells. Rather the energy is stored in other reserve forms like fats, starch, or glycogen. In addition, some species store energy in special forms. The vertebrates employ this reaction (shown on the following page):

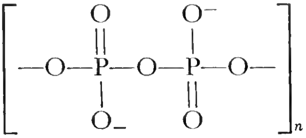


$$^{-}OOC-CH_2-\underset{\underset{\text{creatine}}{CH_3}}{\underset{|}{N}}-\!\!-\!\!-\underset{^{+}NH_2}{\underset{\|}{C}}-NH_2 + ATP \rightarrow$$

$$^{-}OOC-CH_2-\underset{\underset{\text{creatine phosphate}}{CH_3}}{\underset{|}{N}}-\!\!-\!\!-\underset{^{+}NH_2}{\underset{\|}{C}}-NH-PO_3^{=} + ADP$$

in muscle. Some creatine phosphate accumulates during periods of rest and serves as a reserve used during muscular activity. When the muscle is working, the free energy of the creatine phosphate is converted into mechanical energy and creatine reappears for reaction with more ATP. Muscle action in invertebrates involves a related reaction depending on arginine instead of creatine.

Some cells, yeast being the best known, store a complex material called metaphosphate. The linkages resemble those of pyrophosphate and may be represented thus:



$$\left[-O-\underset{\underset{O_-}{|}}{\overset{\overset{O}{\|}}{P}}-O-\underset{\underset{O}{\|}}{\overset{\overset{O^-}{|}}{P}}-O-\right]_n$$

The formation of this grouping from ordinary orthophosphate, $HOPO_3^{=}$, requires free energy, $\Delta F = +10{,}000$ cal. Hence metaphosphate contains a large potential store of chemical energy for use during periods of starvation or any other stress.

Utilization of Energy from Metabolism

Energy transfers of the various types provide for the utilization of the chemical energy of the foods or substrates consumed. The overall efficiency of this utilization is a matter of concern in the economics of food sources and the selection, development, and processing of food materials. These problems all become acute in dense populations where the competition for food is severe. Given an equal food supply, the most efficient organism will tend to survive the stresses of starvation.

A biochemical understanding of the situation is difficult to reach at the level of the metabolic reactions involved because of the complexity of metabolism and incompleteness of the knowledge of the reactions. Nevertheless, some estimates of efficiency have been made. Probably vertebrates do not differ much in this regard, so selection must occur on other bases. Undoubtedly there are differences between vertebrates and other groups but the magnitudes are uncertain. In most cases the efficiency seems to be surprisingly high.

A typical animal converts glucose to carbon dioxide and water during its normal respiration in the presence of oxygen according to the overall reaction:

$$C_6H_{12}O_2 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad \Delta F = -688{,}000 \text{ cal.}$$

At various individual steps in this process, sizable transfers of free energy occur. Some of these form ATP directly from ADP. Other reactions yield DPNH and the like, which later transfer their energy to ADP, forming ATP. There are believed to be about 40 molecules of ATP thus formed per molecule of glucose completely oxidized. However, in two early reactions ATP is required, leaving a net gain of 38 molecules. Each of these molecules has available about 10,500 cal. for the needs of the animal, representing a total of approximately 400,000 cal. or about 60 per cent of that theoretically available from the glucose.

Some of the remaining energy is converted to heat and lost to the environment. However, a part may be converted to heat used in driving some of the processes requiring low-level energy. This latter probability thus raises the efficiency still higher. On the other hand, some of the energy funneled through ATP is lost as heat during subsequent reactions and reduces the efficiency. These effects cannot be evaluated at present, so the overall efficiency cannot be estimated. Nevertheless, it is expected to be fairly high.

Under other conditions some organisms metabolize glucose incompletely by a process called fermentation:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH \qquad \Delta F = -50{,}000 \text{ cal.}$$

One or more compounds other than ethanol may be formed as byproducts, depending upon the species and the environment. While the nature of the products alters the free-energy change, the energies available from the reactions of this type are but a fraction of the energy derived from the complete oxidation of glucose. In the instance above a net gain of only two high-energy molecules equivalent to about 21,000 cal. is observed.

On this basis then, 15 to 20 times as much glucose must be metabolized by a fermentation mechanism to provide energy equal to that from the respiration of glucose. Therefore, given equal opportunities otherwise, fermenting species would be hard put to compete with respiring species. This expectation seems to be fulfilled. However, respiration requires a supply of oxygen, and not all environments contain this gas. In sewage, swamps, certain soil zones, heaps of decaying biological matter, intestinal tracts of animals, or any place having limited contact with air but densely populated with metabolizing cells, there is little or no oxygen. Hence respiration cannot serve to supply energy, and only the mechanisms of fermentation operate. Thus fermentation permits life in otherwise unfavorable situations. The metabolism of the higher plants and animals is of the respiratory type, and these species depend upon oxygen. Certain microorganisms called anaerobes ferment their food materials and require environments very low in oxygen. Others can operate either way, respiring when oxygen is present and fermenting when oxygen is absent. Organisms of this last type are termed facultative on the basis of their diverse capabilities.

METABOLIC OXIDATIONS

The free energy of foods becomes available to a cell as a result of a series of oxidation-reduction reactions. In respiration the cell catalyzes the stepwise oxidation of glucose to carbon dioxide and water in the presence of oxygen, which is reduced during the same course of events. Thus oxidation and reduction are paired, one reactant undergoing one of these changes and another reactant the other.

Consideration of the overall reaction of a typical fermentation is a little more complex on the surface. The fermentation of glucose actually oxidizes part of the molecule to carbon dioxide and reduces the rest to ethanol, for example. Thus the two changes are again paired. If fermentation is indicated by the individual reactions, there are always at least two reactants, one being oxidized and one reduced. Hence the commonly used term, *metabolic oxidations,* must always imply the simultaneous occurrence of reductions.

Oxidation and Reduction

These reactions may occur as the result of one of several different changes in the arrangement of the valence electrons of the substances affected. For the present purpose, oxidations may be most simply considered as involving one of the following changes:

1. Loss of electrons by transfer from one molecule or ion to another with the formation of a new electrovalent bond as in

$$\mathrm{Na} + \mathrm{Cl} \rightarrow \mathrm{Na}^+ + \mathrm{Cl}^-$$

2. Loss of hydrogen atoms by a molecule or ion as in

$$C_2H_5OH + DPN^+ + H_2O \xrightarrow[\text{alcohol dehydrogenase}]{Zn^{++}} CH_3CHO + DPNH + H_3O^+$$

3. Gain of oxygen atoms by a molecule or ion as in

$$CH_3CHO + \tfrac{1}{2}O_2 \rightarrow CH_3COOH$$

In every case the oxidation is accompanied by reduction, which for simplicity may be regarded as the opposite of oxidation, namely, a gain in electrons, a gain in hydrogen atoms, or a loss of oxygen atoms.

The second example deserves some special mention since DPN^+ gains one electron and one hydrogen atom. Usually hydrogen atoms are transferred in pairs in biological reactions. In this case ethanol loses two hydrogen atoms, one going to the DPN^+, while the other splits into its electron and proton. The electron is acquired by the DPN^+ while the proton is set free into the medium and combines with a water molecule. The structural details are shown on page 140. Not all biological reactions are oxidation-reduction reactions. But those that are may be identified by the application of these three criteria.

Oxidation of Foods and Substrates

Some of the substances assimilated from the environment may be incorporated directly into the structural and functional machinery of the cell. Others are modified first and then used for these same purposes. Still other foods are oxidized to provide all necessary energy. This last function of foodstuffs forms the subject of present concern.

In order to classify the information, the overall change in the substrate or food is arbitrarily subdivided as follows:

1. The food is assimilated or digested. Macromolecules are split, usually by hydrolysis, into molecules small enough to pass through cell membranes. These digestive processes are given special consideration later.

2. These smaller molecules undergo a series of reactions leading to still smaller molecules. Many carbohydrates go by a common pathway to pyruvate. This widespread series of reactions has come to be called glycolysis.

3. Pyruvate (ionized pyruvic acid) and several other compounds then undergo additional reactions. In plants and animals these reactions form a cyclic series known as the citric acid cycle, the Krebs

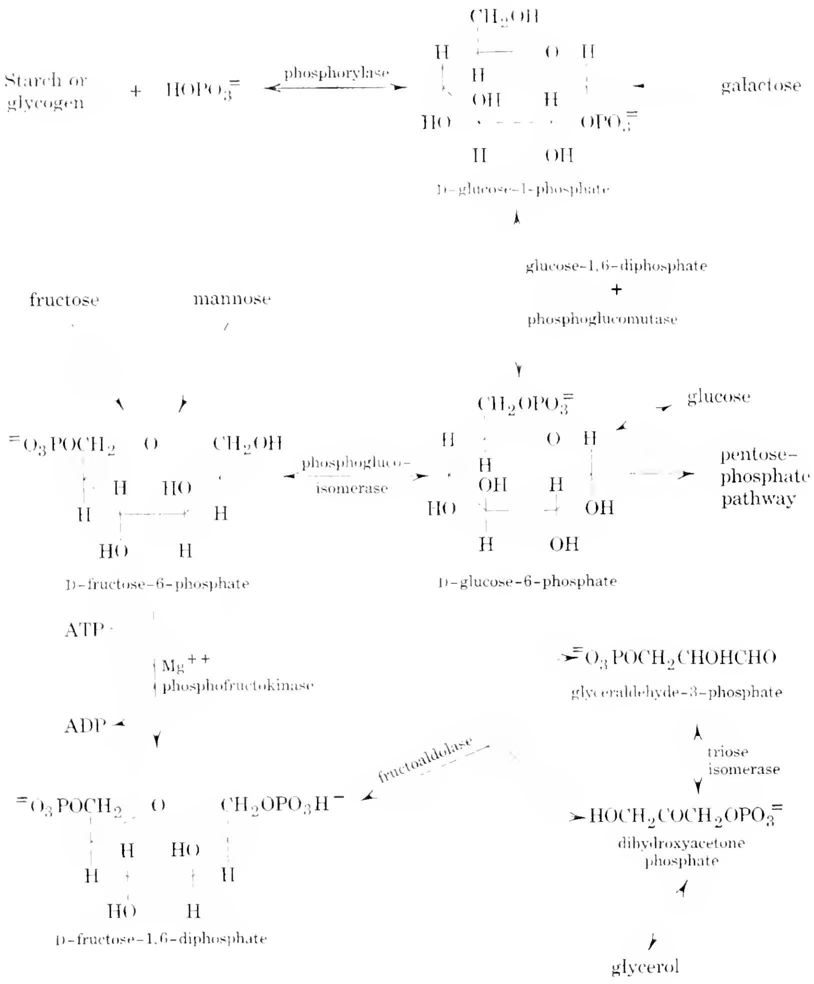
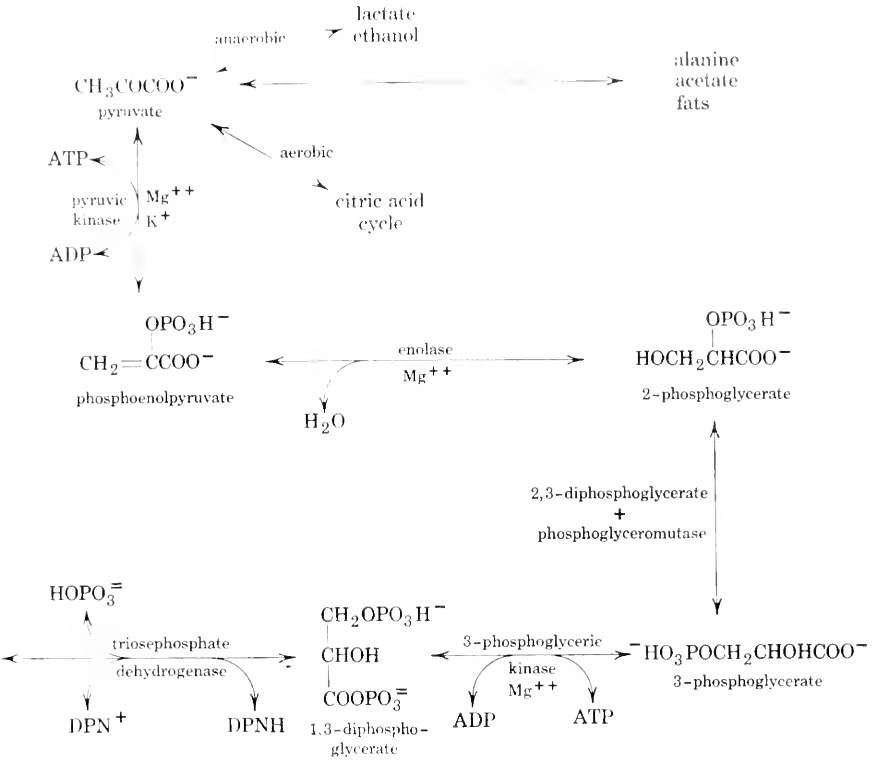


FIGURE 7–1. The reactions of glycolysis as written in 1959.

cycle, or the tricarboxylic acid cycle which yields the carbon dioxide characteristic of respiration.

4. The citric acid cycle also yields DPNH and related reduced materials which transfer electrons to a series of electron acceptors, forming ATP along the way.

5. Finally, the last of the organic electron acceptors transfers electrons to oxygen in the presence of protons from hydrogen ions, water being formed. This ultimate step is the only one utilizing molecular oxygen and is called the terminal oxidation.

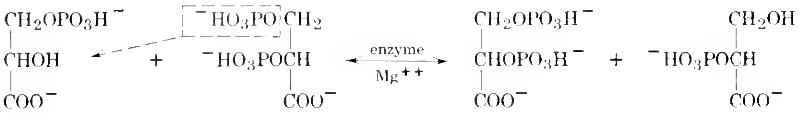


All except the first of these five divisions are illustrated and discussed in the following individual sections.

Glycolysis. The biological reactions grouped under the heading of intermediate metabolism serve to transform a variety of carbohydrates into pyruvate, certain related low-molecular-weight substances, and carbon dioxide. The carbohydrates concerned include those assimilated from the environment and those stored as reserves in the cell during times of plenty.

Although there are several special branches and one known alternate route, our attention will be focused for the present on the pathway believed to be the primary sequence in plants and animals. These reactions (Figure 7–1) are collectively called glycolysis from their role in the metabolism of glycogen. The known ramifications of glycolysis are indicated here by arrows and are summarized later (see pages 214 to 223). Most of these connections require more than one step. Arrows with points at both ends signify that the process is known to be reversible and the actual direction depends upon the supply and demand of reactants and products. As usual the ionizable compounds are shown in a form existing under physiological conditions.

These reactions are all enzymatic, catalyzed by the enzymes named. The known cofactors are also indicated. Two of these substances have not been discussed previously, and both function in the same way.



3-phosphoglycerate 2,3-diphosphoglycerate 2-phosphoglycerate

This mechanism provides for the transfer of the phosphate group without consuming 2,3-diphosphoglycerate. Glucose-6-phosphate is formed from glucose-1-phosphate in the same way, with glucose-1,6-diphosphate as the cofactor and a similar transfer of phosphate.

The interconversion of glycogen or amylopectin and glucose-1-phosphate requires a step not shown because of the branches in these polysaccharides. An enzyme, 4,6-transglucosidase, reversibly shifts the branches from the 4 to the 6 positions, where they can be phoshorylated in the reaction shown.

Anaerobic processes yield lactate, ethanol, and related compounds from pyruvate as indicated. In air much of the pyruvate enters the citric acid cycle. The remainder is converted to acetate, fats, and alanine in either the presence or the absence of oxygen.

The citric acid cycle. This group of reactions (Figure 7–2) provides for the further utilization of pyruvate by respiration, converting the carbon atoms to carbon dioxide. Most of the energy arising from the respiration of plants and animals is derived ultimately from the oxidations and reductions of this cyclic process. For purposes of convenience, pyruvate is shown on the inside of the circular array of reactions.

There are still differences of opinion about several of these reactions, especially some of the more complex transformations. Continuing research will doubtless produce some revisions if the past is any index. Nevertheless, the general form of the citric acid cycle seems soundly established.

The isocitrate to oxalosuccinate conversion involves a different coenzyme than any considered heretofore. Written in the oxidized and reduced forms as TPN+ and TPNH, respectively, the structures correspond to DPN+ and DPNH. The name triphosphopyridine

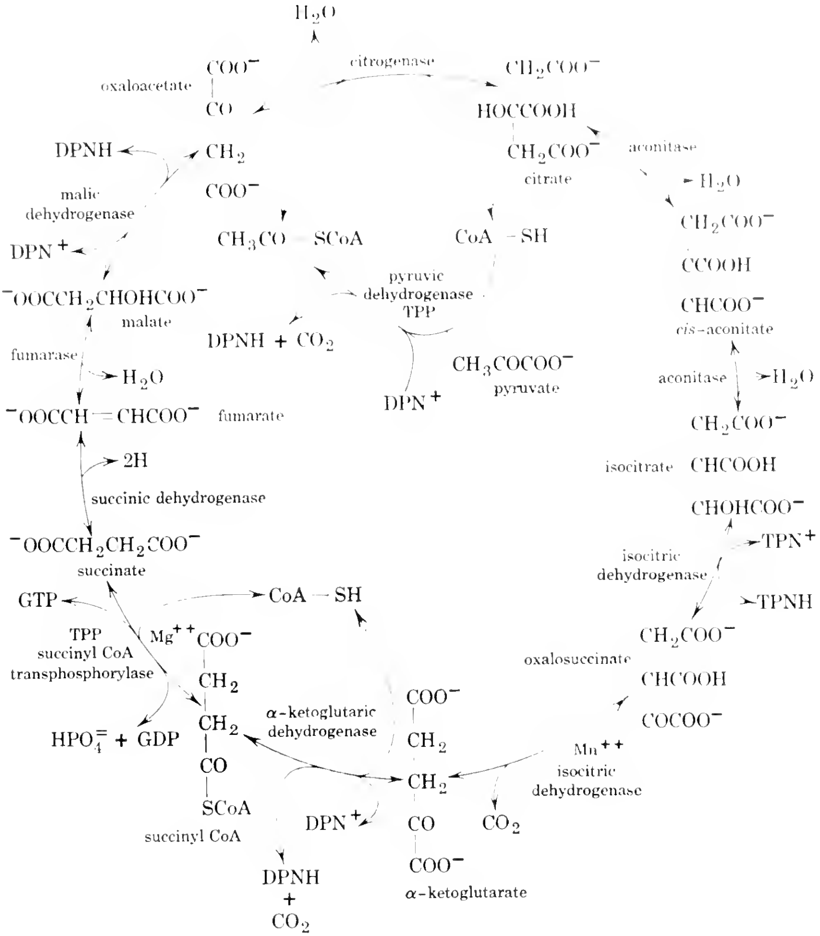


FIGURE 7–2. The Krebs tricarboxylic acid cycle as written in 1959. GTP and GDP represent guanosine triphosphate and guanosine diphosphate, respectively, compounds similar to ATP and ADP but containing guanine instead of adenine. TPP represents thiamine pyrophosphate (page 330), known also as cocarboxylase, and probably acts in combination with a second coenzyme called lipoic acid in the two processes indicated.

nucleotide denotes the presence of a third phosphate group which is attached to the second position of the ribose attached in turn to the adenine. Otherwise DPN^+ and TPN^+ have the same structures.

Each hexose unit metabolized by the pathway of glycolysis yields two molecules of pyruvate. Each pyruvate in turn yields three molecules of carbon dioxide, three of DPNH, and one molecule of TPNH by the reactions of the citric acid cycle. The energy thus accumulated in the DPNH and TPNH is made available by the reactions of the next section. Whenever the supply of hexoses exceeds that required for immediate needs, some of it is metabolized as far as acetyl coenzyme A, then converted to fatty acids. The proteins required for growth and tissue repair depend in part upon three key amino acids derived directly from the pyruvate, α-ketoglutarate, and oxalacetate. The resulting amino acids are alanine, glutamic, and aspartic acids. These various connections with the citric acid cycle are discussed later.

Electron transfer. The fourth and fifth stages in the oxidation of foods and substrates as outlined on page 167 involve the transfer of electrons from DPNH and TPNH through a series of electron acceptors to oxygen. During these reactions three molecules of ATP are formed from ADP and inorganic phosphate for each DPNH oxidized by this route. The present knowledge of the reactions in this system is quite limited, and probably there are differences in different species. However, the steps known for the higher plants and animals may be similar down to the point of transfer of electrons to oxygen. These oxidations can be summarized as follows, starting with either TPNH or DPNH:

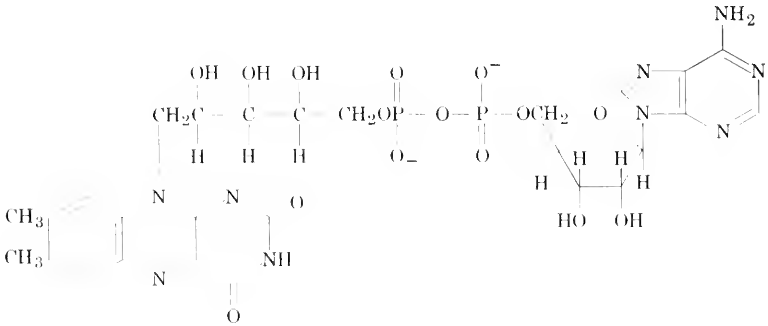
$$\mathrm{TPNH + DPN^+ \leftrightarrow TPN^+ + DPNH}$$

$$\mathrm{DPNH + FAD + H_3O^+ \leftrightarrow DPN^+ + FADH_2 + H_2O}$$

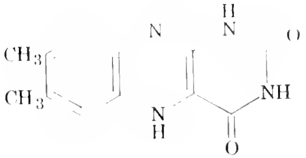
$$\mathrm{FADH_2 + 2\ cytochrome \cdot Fe^{+++} + 2H_2O \leftrightarrow}$$
$$\mathrm{FAD + 2\ cytochrome \cdot Fe^{++} + 2H_3O^+}$$

$$\mathrm{4\ cytochrome \cdot Fe^{++} + 4H_3O^+ + O_2 \leftrightarrow 4\ cytochrome \cdot Fe^{+++} + 6H_2O}$$

FAD = flavine adenine dinucleotide and has the structure



On reduction the lower left-hand portion of the molecule gains hydrogen atoms according to



The term *cytochrome* denotes a complex protein with a prosthetic group belonging to the class called porphyrins and in the present case contains iron. The iron undergoes reversible oxidation-reduction in the presence of enzymes called cytochrome reductase and cytochrome oxidase.

The reactions all show negative free-energy changes totaling about 50,000 cal. Of this chemical energy, about 31,000 cal. is known to be transferred when three molecules of ATP are formed. The fate of the remaining energy is unknown, and the particular reaction stages yielding the ATP are still not known. Moreover, there may be more than one kind of cytochrome serving as electron carriers in series. But in spite of prolonged investigation little has been learned of the sequence of events in cells.

Terminal oxidations. The last step of the above series is the only one mentioned so far that involves molecular oxygen as the oxidizing agent. This reaction and others utilizing oxygen are the terminal oxidations of respiration. When the last cytochrome of the sequence is oxidized by oxygen in the presence of hydrogen ions and an enzyme, cytochrome oxidase, water is formed. The free-energy decrease is thought to be about 25,000 cal., an unusually large amount for a single biological reaction. There must certainly be some mechanism for transferring at least part of this energy to useful compounds, and there may be intervening steps allowing the transfer of energy in smaller quantities.

Other terminal oxidations are known in plants and animals, but the extent to which these reactions function in living cells is still in doubt. They do play roles in various abnormalities and will be discussed later in connection with the metabolism of the two kingdoms concerned. Microorganisms appear to possess considerable diversity in their terminal oxidations, but again most of the details have not been worked out.

The present chapter has summarized part of the information on metabolism, being concerned only with some of the metabolic systems common in higher plants and animals. More details appear in the chapters on plant and animal metabolism. No effort is made to survey the special pathways of the diverse microorganisms.

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Kinetics and Thermodynamics in Biochemistry. H. G. Bray and K. White. Academic Press, New York, 1957.

PLANT
BIOCHEMISTRY

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part 2

Plant structure and composition

8

The mature plant is made up of distinct organs known as roots, stems (stalks or trunks and branches), leaves, and flowers or seeds surrounded by a fleshy or non-fleshy covering. In some plants underground, or subterranean, stems enlarge and serve as food-storage organs for the plant. Through the process of selection and by the application of the science of genetics man has developed various plant types for the production of fruits, grains, potatoes, and vegetables.

THE CELL

The basic unit of all these organs of the plant is the cell. The cell is the seat of the physiological processes involved in plant metabolism (Chapter 9) and reproduction. A representative plant cell may be considered as consisting of a mass of protoplasm containing a vacuole. The protoplasm is in turn surrounded by the cell wall.

The Vacuole

The vacuole consists largely of a water solution of inorganic anions and cations and low-molecular-weight organic substances. Included among the latter are sugars, amino acids, organic acids, and the water-soluble anthocyanin and flavone pigments. The vacuole may be considered a transient depot for these organic and inorganic components, as well as a reservoir for water required for osmotic regulation of the cell.

Protoplasm

The protoplasm of the cell contains essentially all the biological catalysts, the enzymes, that are involved in the complex series of reactions which we call life. Protoplasm is differentiated into *nucleus* and *cytoplasm*.

Nucleus. Most cells contain a single nucleus, a spherical or ovoid body which is denser than the surrounding cytoplasm. The body of the nucleus is made up of a dense jelly-like mass of complex chemical composition known as the *nuclear gel* or *karyolymph* and the *chromatin*. Chromosomes, the deoxyribonucleic acid-rich components responsible for hereditary direction in the organism, appear to originate from the chromatin. In only a few cases is it possible to see chromosomes in the non-dividing nucleus, so it is difficult to make definite statements as to the chromatin-chromosome relationship. Current thought is that during the interphase (interval between cell division) the chromosomes exist in an extended swollen state, filling the whole nucleus evenly. Chemically *chromatin* is composed of deoxyribonucleic acid associated with histone and non-histone protein. The enzymatic activity associated with the nucleus is not well defined at the present time. In addition to chromatin, many cells contain one or more nucleoli. Although the function of the nucleolus is not definitely established, the suggestion has been made that these ribonucleic acid-rich bodies are synthesized in the nucleus and are infiltrated through the nuclear membrane into the cytoplasm. There they function in biological syntheses.

Cytoplasm. The cytoplasmic portion of the cell is less dense than the nucleus. It is not a homogeneous entity but rather consists of a fluid portion containing suspended particulate matter. A detailed description of all the types of formed matter found in the cytoplasm is not within the scope of this book. However, a brief discussion of certain of them is pertinent.

The palisade cells and spongy parenchyma (see Figure 8–1) of higher plants contain green-colored, spherical, ovoid, or disc-shaped bodies, 3 to 10 μ in diameter and 1 to 2 μ in thickness, called *chloroplasts*. The number and size of these bodies vary with the species of the plant and the conditions under which the plant is grown. The chlorophyll appears to be concentrated in bodies known as *grana* which are embedded in a colorless plasma or stroma. In composition the grana are largely protein, lipide, chlorophyll, carotenoid, and

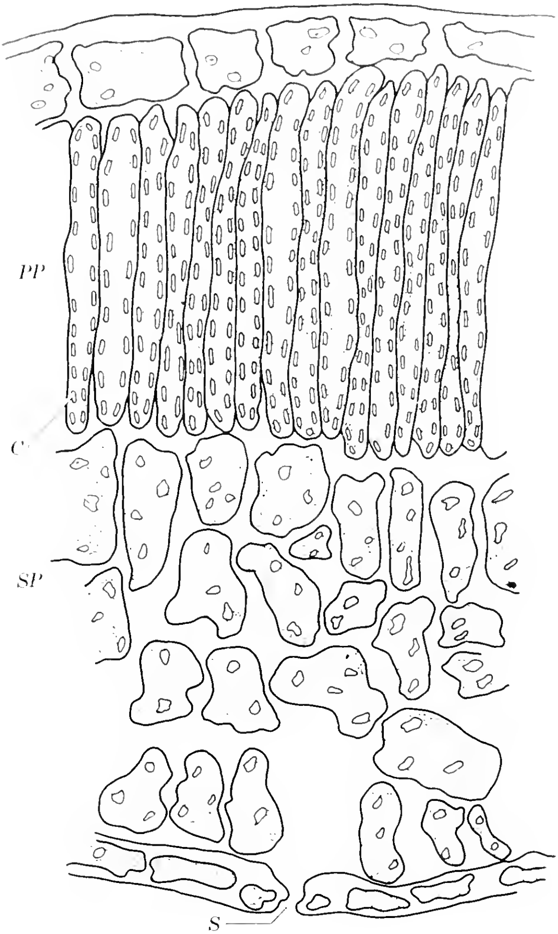

FIGURE 8–1. Cross section of a portion of a leaf, showing the palisade parenchyma (*PP*) and spongy parenchyma (*SP*) containing the chloroplasts (*C*). The stoma (*S*) permit the exchange of gases with the surrounding atmosphere.

ash. Chloroplasts perform an important role in the photosynthetic process (Chapter 9).

Another group of very small bodies, *mitochondria,* is found to be almost universally present in the cytoplasm of plant and animal cells. The mitochondria are small granules about 1 μ in diameter. They are particularly rich in enzyme activity associated with the aerobic oxidation of metabolic intermediates and with oxidative phosphorylation (Chapter 9). The biological activity of the mitochondrial fraction recently has been studied very extensively and is an excellent example of the association of biocatalysts and cofactors within discrete cellular areas. This organization leads to efficient function at maximal local substrate concentrations. Mitochondria contain relatively high amounts of diphosphopyridine nucleotide, triphosphopyridine nucleotide, coenzyme A, flavinadeninedinucleotide, diphosphothiamine, lipoic acid, pyridoxal phosphate, folic acid, and biotin. A multitude of cellular reactions depend upon the presence of such cofactors.

The cytoplasm also contains submicroscopic particles, visible under the electron microscope, called *microsomes.* Microsomes are rich in ribonucleic acid. The activity of this fraction has been associated with nucleotide and protein synthesis as well as with fat metabolism.

The separation of the above-named cytoplasmic components and nucleus can be accomplished by grinding the cells in a suitable buffer with the proper osmotic properties, that is, 0.25 to 0.90M sucrose, followed by centrifugation at different velocities. Low-speed centrifugation sediments the heavier fragments, such as cellular debris. At higher speeds, that is, 5,000 to 21,000 × G,* the mitochondria are sedimented. Prolonged centrifugation in an ultracentrifuge at 60,000 to 80,000 × G permits the recovery of the microsomal fraction. The supernatant remaining after removal of the microsomes contains the soluble enzymes readily extracted from the cells by buffer solutions. Most of the enzymes associated with the glycolysis pathway are associated with this fraction. For example, 90 to 100 per cent of the phosphoglucomutase, hexosediphosphatase, and aldolase activities is recovered in the supernatant.

Starch granules, oil vacuoles, and chromoplasts of various types may also be found in the cytoplasm in large quantities, particularly in cells of organs with special functions, such as storage organs or flower petals.

Cell Wall

The cell wall forms a more or less rigid encasement for the protoplasmic constituents of the cell. The rigidity of the cell wall depends on the age of the plant part. In young tissues the cell wall consists of a single, thin, non-rigid layer. In older tissues it contains two or more thickened layers forming a very rigid structure. The cell wall consists primarily of polysaccharides and polysaccharide derivatives. In some species only a single polysaccharide is present, although in

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* G represents the centrifugal force exerted on the particle expressed in gravitational force units.

most cases a number of these high polymers are involved in the formation of the cell covering. These polymers include such components as cellulose, pectic materials, hemicellulose, and pentosans. Cell walls of the woody and stemmy portions of the plant contain an additional strengthening agent, a complex organic substance called lignin. Although the cell wall is rigid, it is by no means impervious to water. Pores in the structure permit the ready passage of smaller molecules from the protoplasm of one cell to that of a neighboring cell. Plant physiologists believe that the protoplasmic membrane separating the cytoplasm from the cell wall constitutes the semipermeable membrane rather than the essentially inert cell wall. There is little or no evidence pointing to the existence of extensive enzyme activity in the cell wall. Once laid down, the wall is relatively inert and the constituents remain outside the active metabolic pool.

It is hoped that this brief discussion of the plant cell demonstrates that this unit of the plant is not merely a homogeneous bag of enzymes and substrate. Each structural entity of the protoplasm carries its complement of enzymes in a highly organized manner. The proper functioning of a cell as a whole requires synchronization of the enzyme systems of the various discrete units as well as the soluble enzyme systems. Investigations of the interrelationships of these various cytoplasmic components occupy a large portion of the research effort at the present time.

STRUCTURAL MATERIALS

The various chemical constituents of the plant can be considered from the standpoint of utility to the cell, that is, structural materials, food reserves, metabolic machinery, and special substances. This last group includes such items as essential oils, flower pigments, alkaloids, and other chemical entities for which no present function is known. As the science of biochemistry advances, many of these items will undoubtedly be reclassified into one of the above functional classes.

Polysaccharides constitute approximately 75 per cent of the dry weight of higher plants. Most of these polysaccharides are components of the cell wall. In young cells the quantity of such structural materials is very low. On the other hand, in mature cells the walls are often so thick as to leave only a small cavity within the cell. Such cells frequently die and then serve only in supporting and protecting the attendant softer tissues.

Cellulose

The principal polysaccharide found in the cell wall of higher plants is cellulose (Chapter 3). Generally it is found in association with other polysaccharides, lignin, and inorganic silicates. However, cellulose may be found in almost pure form in cotton and ramie bast fibers. Plant fibers are bundles of high-molecular-weight, linear cellulose molecules held together by physical chemical forces at intervals along the chains. The cellulose fiber, therefore, consists of repeating crystalline areas interspersed with non-crystalline (amorphous) areas. X-ray analysis has been used in determining the structure of the crystalline regions called micelles. From X-ray data it has been possible to calculate the volume of the unit micelle (670 Å.^{3}) and to determine the average glucose content. Each micelle contains an average of four glucose units. From this information and from the calculated micellular dimensions (10.3 × 7.9 × 8.3 Å.), a structure for the crystalline region of cellulose has been proposed as shown in Figure 8–2.

The unit cell has a length of two D-glucose units (one cellobiose unit). Thus each cell contains an average of two cellobiose units with the corner units shared with the neighboring micelles. Note also that

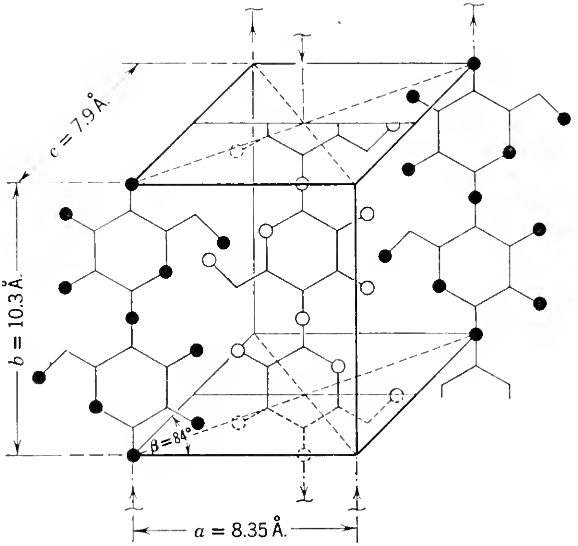


FIGURE 8–2. Unit cell of cellulose. (From *Cellulose and Cellulose Derivatives,* Volume V, Part 1, of High Polymer Series, copyright 1954. Published with permission of Interscience Publishers, New York.)

the chains are directed alternately with the glycosidic linkages oriented in opposite directions. Within the crystalline regions the closely packed cellobiose units of the cellulose chains are held together by hydrogen bonding, presumably involving chiefly the —OH groups of carbons 2 and 6.

The cellulose molecules of one micelle are interwoven with those of others in the amorphous region. They may be incorporated into different micelles in the succeeding crystalline regions. The fabric-like web which results from this pattern is responsible for the strength of the cellulose fiber making up the cell wall.

Water absorption in the cellulose fiber is confined almost entirely to the amorphous regions. Swelling in hydrated cellulose is perpendicular to the linear chains, with very little elongation of the fiber. In addition, the amorphous areas are more subject to chemical and enzymatic attack than the crystalline areas.

Although the cellulose once laid down in the cell wall does not appear to be utilizable by the plant itself, nature has provided a large number of enzymes found in microorganisms that are capable of hydrolyzing cellulose at a rapid rate. If this were not the case, the surface of the earth would soon be covered with dead plant tissue, such as annual plants, grasses, leaves, and trunks of trees. Such an accumulation would soon lead to depletion of the CO_2 reserves (page 68). Many bacteria produce extracellular enzymes capable of hydrolyzing cellulose, the reaction proceeding as follows:

$$\text{Cellulose} \xrightarrow{\text{cellulase}} \text{cellodextrins} \rightarrow \text{cellobiose} \xrightarrow{\text{cellobiase}} \text{glucose}$$

Many workers feel that this process is operative in rumen fermentation (Chapter 17) and leads to the utilization of quantities of cellulose in the roughage in the multistomach animals.

Hemicelluloses and Other Cell-wall Polysaccharides

Interlaced with the cellulose web in the primary and secondary cell walls are numerous other polysaccharides characterized chiefly by their solubility in 17.5 per cent sodium hydroxide solution and their ready hydrolysis in hot dilute acid. This group of compounds was given the name *hemicellulose* by early investigators because of the presumed relationship to cellulose. The absence of any direct relationship has been evident for some time, but the name, once applied, has remained unchanged. The monosaccharide component of hemicellulose varies with the species involved and with age within the species. The hemicellulose contents based on the dry weight of the plant residues vary from less than 0.5 per cent in cotton fiber to 40 per cent in hybrid corncobs. Table 8–1 lists reported cellulose, hemicellulose, and lignin contents of some of the agricultural sources of cellulose.

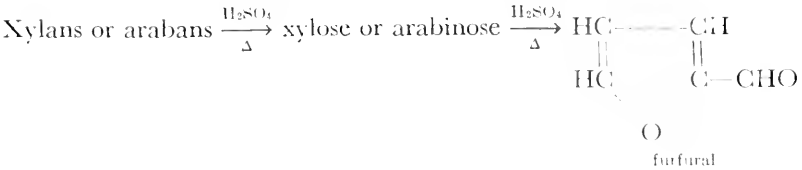
TABLE 8–1. Cellulose, Hemicellulose, and Lignin Contents of Plant Tissues

Tissue	Cellulose, %	Hemicellulose, %	Lignin, %
Hardwood			
Aspen	51	21	18
Post oak	42	20	22
Softwood			
Douglas fur	54	13	27
Black spruce	49	17	27
Redwood	36	10	31
Annual plant residues			
Hybrid corncobs	46	40	12
Open pollinated corn	43	31	19
Wheat straw	50	25	20
Flax straw	45	25	29

By far the most common polysaccharides in the hemicellulose mixture are the xylans. These pentosans are composed of D-xylose units coupled by 1 → 4 β linkages with or without branching. Xylans occur in the secondary cell walls of practically all land plants. In some of them, xylans may contain, or be closely associated with, L-arabinose. Pentosans of esparto grass are reported to contain 1 arabinose unit per 18 xylose residues.

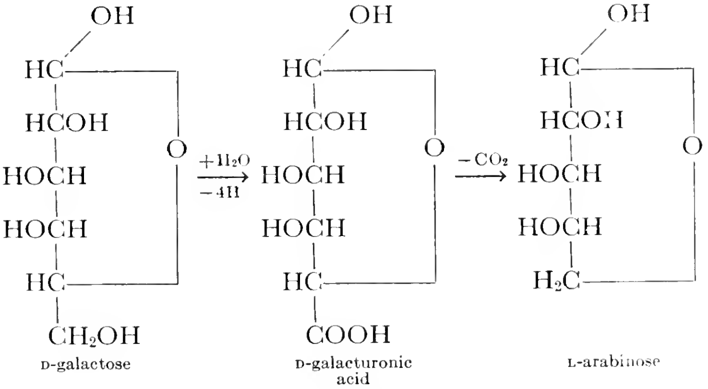
In addition to xylans and arabans, the hemicellulose fraction contains polysaccharides composed of D-glucuronic or D-galacturonic acids in combination with other pentoses and hexoses. Some of the hardwood hemicelluloses are reported to contain as high as 18 per cent uronic acid.

The hemicelluloses of oat hulls or corncobs are the point of origin of the important industrial chemical, furfural. Development of production methods for furfural can be attributed to an accumulation of oat hulls and the resulting disposal problem. Chemists at Quaker Oats Company found that they could produce furfural in commercial quantities by digesting the hulls with sulfuric acid followed by distillation. After developing the production methods, it was then necessary to find a market for the product. Today furfural is an important industrial solvent, particularly in petroleum refining, as well as an intermediate in chemical reactions. Its production may be pictured as follows:



Pectic Substances

Pectic substances form an important group of compounds occurring in the primary cell wall and the intercellular layers of land plants. Structurally, the pectins contain an admixture of three polymers: (1) D-galacturonic methyl ester, (2) D-galactans, and (3) L-arabans. The close association of these three polymers has led some workers to suggest that the uronic acid polymer arises from the D-galactan through the oxidation of the primary alcohol (number 6 carbon). Decarboxylation of the uronic polymer could give rise to the L-araban polymer.



The theory, while attractive, is untenable because: (1) the araban in pectin contains arabinose with the furano ring, while the galactose is pyranose in character, and (2) the galactan polymer is linear as compared with the highly branched araban structure. The synthetic routes involved in the building of the pectin components remain obscure.

Young growing fruits first lay down protopectin, a water-insoluble pectic substance of high molecular weight. As the fruit ripens, the protopectin is hydrolyzed to the smaller water-soluble pectins by the enzyme protopectinase. Upon further ripening, the resulting pectin is subjected to attack by two more enzymes, pectases and pectinases. The pectases hydrolyze the ester groups to produce pectic acid, and pectinases hydrolyze the glycosidic linkages to produce uronic acid and intermediate polymers.

Pectin is characterized by its water-binding capacity, particularly in the presence of dehydrating agents such as sucrose. This property is used in the preparation of jams and jellies. Commercial pectin is prepared from apple pumice and from citrus peel. Since enzymic hydrolysis takes place and since pectic acid and the lower polymers resulting from pectinase activity have little or no gelling power, it is important that the quality of the starting material be controlled.

Certain plants produce additional polysaccharide derivatives which are noted for their viscosity in water solutions. Since a number of these are important commercial items, they will be mentioned briefly. They are not components of the cell wall, but rather serve as protective coatings of either seeds or wounded areas.

Gum Arabic

Gum arabic is obtained as an exudate from the bark of the *Acacia* tree of Central Africa. It has a highly branched structure containing D-galactose, L-arabinose, L-rhamnose, and glucuronic acid. Gum arabic is used widely in the food industry to control viscosity and texture.

Gum Tragacanth

Gum tragacanth is produced on a semidesert shrub of the *Astragalus* family in Asia Minor. The exudate is only partially soluble in water. The soluble portion is extracted from the harvested gum and sold as traganth for use in the drug, cosmetic, and printing industries. Again, the function is the control of viscosity and texture.

Mucilages

Mucilages are obtained by water extraction of the seed coat of flax, *Plantago,* mustard, and certain other seeds followed by precipitation with alcohol. As the name implies, a water solution of mucilage produces a sticky, viscous mass. In nature this property probably serves in seed distribution and also as a means of retaining moisture needed for seed germination.

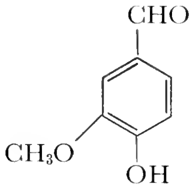
A number of gel-forming substances have been isolated from sea algae. These include the commercially important *agar,* isolated from red algae of the Pacific Ocean, and *carrageen*, recovered from another red algae commonly known as Irish moss. Both these polysaccharides are polygalactansulfate esters of varying molecular weight.

Lignin

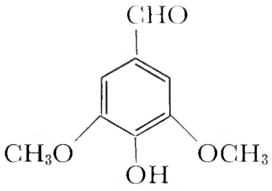
The cell walls of woody parts of plants are strengthened by a complex organic material called lignin. It may occur in a range from a few per cent in some soft woods to 50 per cent or more in some coniferous species (Table 8–1). Lignin is deposited in the interstices between the microfibrils and the micellar strands of cellulose and other components to form a three-dimensional plastic which serves as a cementing substance for the woven pattern of the other components of the cell wall. In wood lignin is located chiefly in the middle lamella with smaller amounts in the primary and secondary layers of the cell wall. In annual plants lignin is found on the surface of the fiber and appears to be less highly cross-linked than in wood. It can be much more readily removed by the chlorination or sulfite process. The question of whether lignin is chemically bound to the neighboring carbohydrates or whether the linkages occur only as hydrogen bonds remains unsolved.

Lignin is an amorphous, yellow or brown substance of indeterminate molecular weight. It contains approximately 63 per cent carbon, 6 per cent hydrogen, and 31 per cent oxygen; the observed values will vary somewhat with its origin and the method of separation. Lignin contains a large proportion of aromatic rings with a high content of methoxyl groups. Complete oxidation yields carbon dioxide and water. If the conditions of oxidation are controlled, it is possible to isolate a variety of aromatic aldehydes and acids as well as small amounts of aliphatic monocarboxylic and dicarboxylic acids. Mild oxidation of spruce wood lignin, as in the presence of nitrobenzene, yields up to 25 per cent vanillin.

Oxidation of lignin derived from hardwoods yields syringaldehyde in addition to vanillin. Lignin has also been subjected to alkali fusion and to dry distillation with the production of small amounts of a variety of aromatic compounds. In addition, small quantities of nonaromatic substances, such as formic, acetic, and oxalic acids, have been isolated.

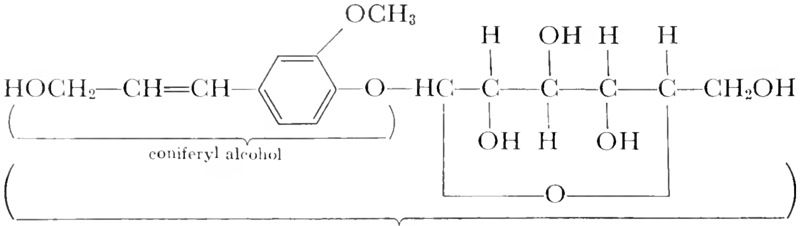


vanillin



syringaldehyde

The high carbon and low hydrogen contents mentioned earlier, and the variety of benzenoid derivatives isolated from oxidized or fused lignin, indicate the aromatic character. Many structural formulas have been proposed for the compound, but conclusive evidence to support any of them is lacking. A relationship between coniferin and lignin has been suggested. Coniferin is a glucoside of coniferyl alcohol and is isolated from the cambial sap of the spruce and other conifers.



coniferin

The free coniferyl alcohol has also been isolated from the sap of pine species. This phenylpropyl structure has been suggested as the central building unit. However, polymerizations of the above compound cannot account for the high degree of cross-linking in lignin and the stability to hydriodic acid. Involvement of such a unit does account for the high vanillin yields on mild oxidation and for the compounds isolated on alkali fusion.

RESERVE MATERIALS

Once formed, the structural materials discussed in the preceding section are quite inert when considered from the metabolic standpoint. Plants produce carbohydrates, lipides, or proteins which serve as reserves during periods of energy surplus. Since plants, unlike animals, provide their own food by way of the photosynthetic process, these reserves are stored (1) in the active cell in amyloplasts, oil vacuoles, (2) in special storage organs for the purpose of providing new vegetative growth, or (3) in the seed.

Carbohydrates

Starch. The most abundant reserve carbohydrate in the plant world is starch. Under favorable conditions it may be found in all the cells of the living plant, usually in grains or granules. The two components of starch, amylose and amylopectin (Chapter 3), vary in proportion in different plants. Some plants produce predominantly amylose; others accumulate a starch which is almost pure amylopectin. Table 8–2 lists the average distribution of the two components in some common starches. It will be noted from the table that not only does the percentage distribution of the two components vary, but so does the size of the molecule. The significance of these size differences to the

TABLE 8–2. Size and Proportion of Amylose and Amylopectin in Some Starches

Plant Species	Amylose, %	Glucose Residues per Molecule	Amylopectin, %	Glucose Residues per Molecule
Tubers, roots or rhizome				
Tapioca	17	980	83	18,600
Potato	22	980	78	...
Lily	34	640	66	18,600
Seeds				
Corn, hybrid	22	490	78	30,850
Corn, waxy	0	...	100	...
Wheat	24	540	76	24,700
Rice	17	...	83	...

economy of the plant is not apparent. The starch granules of the different plants also vary in size from 1 μ to 150 μ in diameter. Potato tubers produce large granules, whereas rice and buckwheat produce very small granules. The size, shape, and markings on the granules are sufficiently characteristic for the microscopic determination of the origin of a starch in food products. There is some evidence that the layering, or stratification, shown clearly in the apparently concentric rings in potato starch, is due to the day and night alternation in rate of formation of the grain.

Amylose can be separated from amylopectin by treating the grains with hot water. The linear amylose diffuses from the swollen granule while the amylopectin fraction remains behind. On standing, a rather pure amylose precipitates in low yield from the solution. A better separation of the two components of starch can be achieved by heating starch with a butanol-water solution under pressure. Upon cooling, an amylose-butanol complex precipitates. Amylopectin is recovered by drying or by alcohol precipitation.

None of these methods provides a commercially feasible method of separation. For many commercial purposes it would be desirable to have a preponderance of one type of polymer rather than a mixture of the two components. The problem of commercial separation has been partially solved by the plant geneticists who have bred strains of corn, rice, and barley yielding only amylopectin. This work was accomplished before World War II and provided a source of starch with good gelling properties at a time when importation of tapioca starch was impossible. Recently the geneticists have also succeeded in producing a high-yielding corn with a very high amylose content. Amylose can be used to prepare transparent films similar to cellophane but with one important difference: amylose films are digestible. Such films will provide an edible coating for meats and various frozen foods.

Crops that are valuable sources of commercial starch include the cereals (corn, rice, and wheat) and the tubers (Irish and sweet potatoes). Of these corn is the most important because it is produced in greater quantities than any other American cereal. Although approximately 3 billion bushels of corn are produced annually in the United States, only about 3 per cent of this amount finds its way into non-food industrial channels. The remaining 97 per cent serves as food for human beings and domestic livestock. Much of the corn utilized industrially is used for starch manufacture, although appreciable quantities are purchased by the fermentation industries.

Sucrose. Sucrose (page 64) is not only a major photosynthetic product and sugar of translocation; it is also one of the principal forms of carbohydrate storage in a number of plants, particularly certain members of the cane family, and in some root crops, for example, sugar beet. Sucrose is found in small quantities in all metabolically active plant tissue. Fruits and juices of many plants contain appreciable quantities of sucrose, as does the nectar of flowers.

The principal uses for sucrose are as foods and candy products. Large quantities are also consumed by the fermentation industry for the production of alcohol, citric acid, lactic acid, and levulinic acid.

Other reserve carbohydrates. Although starch and sucrose are the storage forms of reserve carbohydrates in the major economically valuable plants, a number of other carbohydrates are found in a variety of plants. Members of the Compositae and Graminaceae families accumulate D-fructofuranose polymers in stems, rhizomes or tubers. The fructosans (page 69) are of low molecular weight and readily water soluble.

Legume endosperms accumulate a branched-chain polymer composed of D-galactose and D-mannose as food reserves. The polymer consists of linear chains of mannose linked β-1 → 4, with D-galactopyranose units linked α-1 → 6. Guar is grown as a commercial source of this polymer. The galactomannans are excellent solution thickeners.

Lipides

The term *lipides* includes a variety of organic compounds found in the plant (Chapter 4). In many cases the term is synonymous with "ether extractables," and includes the triglycerides, phospholipides, and waxes as well as the non-fatty-acid-containing substances, resins, resin acids, terpenes (essential oils), and plant sterols.

Fats and oils. Fats and oils are found in the greatest quantity in storage regions of the plants, particularly in the seeds or fleshy coverings of certain seeds, but they occur in all parts of the plant in small quantities. In general, the vegetative organs contain only small quantities of fats. The fat content of leaf tissue varies from 0.5 to 5 per cent in common species. Stems and bark tissue contain similar amounts. Seeds and fruits vary widely in their oil content. Wheat, oats, corn, and barley contain from 2 to 5 per cent oil, whereas the oil seeds may contain up to 60 per cent oil. Most fruits are low in oil; however, the avocado contains about 20 per cent and the olive fruit 50 per cent. Both the total triglyceride content and the fatty acid distribution within the fat or oil vary in different fruits. Table 8–3 shows the oil content and major fatty acid composition of some of the common oil seeds and fruits. It must be remembered that these are average values. Within limits biological variation and varietal differences influence both the oil content and the composition.

In addition to the commodities mentioned in Table 8–3, rapeseed and sesame seed oils constitute important sources of industrial and food oils in some parts of the world. Total world production of vegetable oils for food and industrial purposes is in excess of 25,000,000 tons. Peanut, cottonseed, soybean, coconut, olive, and corn oils are used extensively in the food industry for shortenings, margarines, and

TABLE 8–3. Oil Content and Composition of Some Seeds and Fruits

Tissue and Species	Fat, %, dry-weight basis	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic	α-Eleostearic	Ricinoleic
Nut or seed										
Peanut	45	. . .	. . .	8.3	3.1	56.0	26.0	. . .	. . .	. . .
Cotton (whole seed)	20	. . .	0.5	21.9	1.9	30.7	44.9	. . .	. . .	. . .
Soybean	18	. . .	0.1	9.8	2.4	28.4	50.7	6.5	. . .	. . .
Flaxseed	38	. . .	0.2	5.4	3.5	19.0	24.0	47.0	. . .	. . .
Palm	50	. . .	1.5	42.9	4.7	39.8	11.3	. . .	. . .	. . .
Coconut (copra)	67	46.4	18.0	9.0	1.0	7.6	1.6	. . .	. . .	. . .
Castor bean	48	. . .	. . .	. . .	. . .	7.4	3.1	. . .	. . .	87.0
Tung (kernel)	58	. . .	. . .	5.5	. . .	4.0	8.5	. . .	82.0	. . .
Seed germ										
Corn	35	. . .	0.5	10.0	3.5	33.0	53.0	. . .	. . .	. . .
Fruit										
Olive	30–65	. . .	1.2	15.6	2.0	64.6	15.0	. . .	. . .	. . .

salad oils. Flaxseed (linseed), tung, castor bean, and soybean oils have found extensive application in the protective coating, linoleum, and printer's ink fields. Palm and coconut oils are used extensively by the detergent industry, particularly in the manufacture of synthetic detergents. The fatty acid distribution in the oil is the governing factor in determining the major use, although by means of hydrogenation even the highly unsaturated oils are converted into satisfactory fats for food and industrial purposes requiring greater stability.

Tall oil. Tall oil is an ether-soluble complex isolated from coniferous woods during pulping by the kraft sulfate process. It is a mixture of fatty and rosin acids. After separation from the rosin acids by distillation, tall oil is sold to linoleum, protective coating, and detergent industries in competition with other vegetable oils.

Phospholipides. The phospholipides (phosphatides) represent a group of combined or conjugated lipides containing a phosphoric acid group in the molecule. Representative members are discussed in Chapter 4. Phospholipides are components of vegetable oils such as corn, rapeseed, soybean, linseed, and other plant oils. Plants contain smaller amounts than are normally found in some animal tissues like the liver and brain. It is probable phospholipides are components of all living cells, with the greatest quantities found in tissues rich in fat. Although this lipide fraction appears to parallel fats in quantitative distribution, phospholipides do not occur in dispersed droplets (oil vacuoles). There is good evidence that phospholipides are present in tissues combined with proteins and possibly carbohydrates. In the leaf this fraction appears to be confined to the grana of the chloroplasts. Phospholipides of the oil seeds disappear on germination, as do the other lipides, but at a slower rate. The same statement is true of the leaves, where, under starvation conditions, the triglycerides are utilized at a more rapid rate than the phospholipides. There is some question as to whether the latter compounds should be classified as reserve lipides in the leaf or whether they should be considered to have another function. Some recently published work indicates that certain members of this group may play a role in phosphate transport in the plant. Other members have been implicated in other group transfer reactions in the organism.

Although plants cannot be considered rich sources of the phospholipides, quantities are recovered during refining of food and industrial oils. It is estimated that over 60,000,000 lb. of crude lecithin is available annually as a by-product of the soybean oil refining process. Crude "lecithin" is a mixture of compounds, including lecithin, cephalin, phosphatidic acids, and sulfolipides.

Lecithin is used in the food industry as an emulsifying agent and as an antioxidant in the confectionary trade to preserve the "bloom" and prevent graying of chocolate in hot weather. Lecithin is also used in compounding cosmetics and pharmaceutical preparations. Addition of small amounts to the surface of paints in cans prevents film formation during storage.

Proteins

Although proteins are usually thought of as biological catalysts (Chapters 5 and 6), in the seeds of many plants these important polymers also serve as stored food reserves. Since most of the cereal grains contain only about 10 per cent protein, these seeds depend primarily on the carbohydrate reserves. Seeds of many species of dicotyledonous plants, on the other hand, frequently accumulate larger quantities of reserve proteins in the cotyledons (20 to 30 per cent). Globulins (page 114), which constitute the largest part of dicotyledonous proteins, are extracted from the defatted seed meal with 5 to 10 per cent sodium chloride and may be precipitated by dialysis against water, or by the addition of ammonium sulfate. Crystalline globulins, prepared from hemp, squash, and melon seeds, have molecular weights of 200,000 to 430,000.

Proteins from different vegetable sources, particularly from soybean and cottonseed meals, have found wide industrial application. Plant proteins from different sources are so similar in chemical composition that they can be used interchangeably for many industrial purposes. Ease of isolation and cost of the starting material are the determining factors. The most important industrial uses of proteins are for the production of plastics and adhesives, in coatings for paper products, and in bonding plywood veneers. Artificial textile fibers can be prepared from vegetable proteins but have not been used extensively because of competition from synthetic fibers. Similarly proteins in water-based paints have been replaced largely by synthetic products.

METABOLIC MACHINERY

Proteins

In the preceding section plant proteins were discussed as reserve materials. Associated with the reserve proteins in the seed, and widely distributed throughout the remainder of the plant, are the proteins which serve to catalyze the multitude of reactions involved in the complicated process termed *metabolism* (Chapter 9). Studies on the leaf proteins are not nearly so extensive as those devoted to the seed proteins for the simple reason that the subject is complicated by (1) the instability of leaf proteins and (2) the large number of components involved. Seed globulins are not easily denatured, whereas the leaf proteins are very readily altered by relatively mild treatment.

In discussing cellular constituents, mention was made of centrifugal separation of cellular components. By this means it is possible to investigate separately the protein components of the nucleus, chloroplasts, mitochondria, microsomes, and cytoplasmic fluid. Although the biological activities of these cellular fractions has been studied in some detail (pages 178 to 180), information on the complete spectrum of proteins involved is lacking. Cytoplasmic proteins have been separated into a number of fractions by the use of classical methods. From the known enzymatic reactions of the soluble components it is obvious that each fraction must contain a large number of different enzymes.

The nuclear proteins have also been subjected to extensive investigation. It was once thought that the nucleoproteins were confined to the nucleus, therefore the name. It is now known that, whereas ribonucleoproteins are to be found primarily in the cytoplasm, some occur in the nucleoli. Deoxyribonucleoproteins are the main constituents of chromosomes and are presumably directors of the hereditary process. Ribonucleoproteins are constituents of microsomes and mitochondria. The microsomes are thought to be involved in protein synthesis and thus would serve as the connecting link between the controlling nucleus, the heredity, and the metabolically active cytoplasm.

Metabolic Cofactors

Proteins require the assistance of large numbers of cofactors in the performance of their roles as catalysts. Included in this class are the requisite inorganic elements (page 141), vitamins (page 139), nucleotides (page 120), and the plant growth regulators (page 280). Strictly speaking, the term *vitamin* does not apply to plants since these cofactors are synthesized by the plant; however, the term will be used to avoid confusion.

In general, the vitamin distribution in plants follows the same pattern as in animals. The greatest concentrations are found in the regions of most active vegetative growth or in the centers of reproduction. For example, many of the B vitamins are associated closely with the mitochondria (page 179). The fat-soluble vitamins E and K are closely associated with the lipide and phospholipide portions of the plant. The germ or embryo is particularly rich in vitamin E. Although no definite metabolic role in the plant can be ascribed to these vitamins, there are suggestions that they play a role in electron transport, particularly in the photosynthetic process. The fat-soluble vitamins A and D are not found in plants, although the precursors β-carotene (page 208) and ergosterol (page 94) are present in many plant tissues.

The distribution of the nucleotides in plants follows the same pattern as that of the vitamins, the greatest concentrations occurring in the rapidly metabolizing cells. The same is true of many of the essential minerals. Manganese, zinc, magnesium, potassium, molybdenum, copper, and iron are either components of, or form complexes with, enzyme systems in performing their cofactor roles. Magnesium is a component part of chlorophyll (page 207). Iron and copper are constituents of oxidases and electron receptors (pages 150 and 172). Calcium is found in both the cytoplasm and in many cell walls where it occurs as calcium pectate, a hydrophylic colloid which presumably plays a part in water imbibition by the cell. Calcium pectate acts also as mortar in cementing the cells together. Boron is distributed throughout the plant, although the greatest concentrations are found in the growing tips. The remaining requisite elements have already been described in preceding chapters as constituents of proteins.

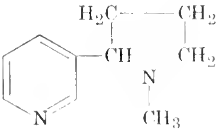
INCIDENTAL AND SPECIAL SUBSTANCES

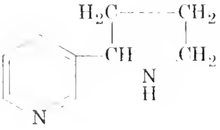
Non-essential Elements

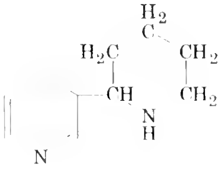
In addition to the above-mentioned essential elements, the plant absorbs many of the diverse inorganic ions found in the soil. Sodium ion is found in all plant tissues grown under usual conditions. Although it cannot be considered essential for growth and reproduction, many plants, such as beets, celery, and cabbage, respond to sodium additions when grown in deficient soils. Silicon and aluminum, two common elements in most soils, are accumulated in small quantities, particularly in the woody or stem tissues. Silicon may serve as a stiffening agent in the cell walls. Cesium and rubidium compete with potassium in the absorption process. Plants grown in soils containing appreciable quantities of these and similar elements contain measurable amounts. In most cases such absorption is inconsequential, both to the plant and to the animal which consumes it; however, selenium provides a well-known exception (pages 390 to 392).

Alkaloids

Many plants throughout the world accumulate organic nitrogen bases called alkaloids. Although a large number of medicinally important alkaloids have been isolated, only in nicotine, nornicotine, and anabasine of tobacco has the plant physiology been studied extensively. Nicotine is the principal alkaloid of tobacco and constitutes 0.5 to 8 per cent or more of the dry leaf. The quantity found in tobacco is a function of variety and type, nutritional status, and growing conditions. Nicotine is synthesized predominantly in the roots and transported upward in the transpiration stem to the leaves. Many workers believe that nicotine, once formed and transported to the leaf, undergoes no further change and remains outside the metabolic pool. Recent work with C^{14}- and N^{15}-labeled nicotine has indicated that this is not entirely true. The tracer element was found in the amino acids and other nitrogenous constituents in plants supplied with tagged nicotine.


nicotine

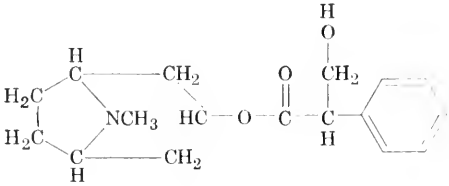

nornicotine


anabasine

Nicotine is converted to nornicotine in the leaf of tobacco. In most varieties and types only a small portion of the nicotine is converted to nornicotine, but in a few selected varieties nornicotine predominates.

Anabasine is found in small to negligible quantities in most tobaccos of commerce, although it is the principal alkaloid of the tobacco species *Nicotiana glauca*. Unlike nicotine, this alkaloid is produced in both the roots and the leaves.

The solanaceous plants produce a number of alkaloids of medicinal value. Atropine, a drug used to dilate the pupil of the eye, is produced by a number of plants of the belladonna family. Most of the drug is


atropine

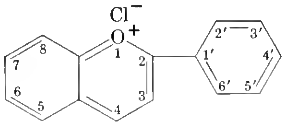
imported from Central Europe. Like nicotine, atropine is also synthesized in the roots and later moved into the stems, leaves, and seeds.

Space will not permit listing all the alkaloids of medical value. Many of the structures are extremely complex and therefore difficult to establish with certainty. The common medical compounds cocaine, codeine, quinine, strychnine, and morphine are alkaloids.

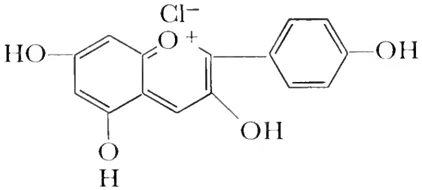
Alkaloids have no known function in plants. The suggestion has been made that they represent nitrogen excretory products, or that they are by-products of nitrogen metabolism. Why these by-products should be formed in such quantities in the root and transported to the leaves for storage remains to be explained.

Flower Pigments

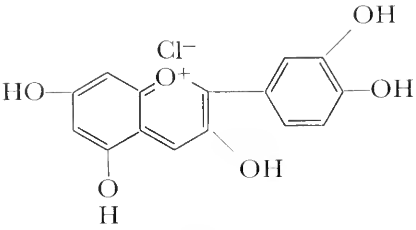
The numerous blue, purple, magenta, and nearly all the red flower, stem, fruit, and leaf pigments belong to a group of glycosides (page 72) designated as anthocyanins. The water-soluble yellow pigments in flowers, stems, and roots are generally flavones (anthoxanthins) and may occur as glycosides or uncombined as the free pigment. These pigments are usually freely soluble in the cell sap, although some occur in an amorphous or crystalline state, as in species of *Delphinin.* The chemistry of these compounds has been studied in considerable detail. Anthocyanins are all derivatives of 2-phenylbenzopyrylium salt. The principal difference between various members is the degree of hydroxylation of the phenyl ring and the presence or absence of methyl esters. Pelargonidin, the pigment of scarlet pelargonium, orange-red dahlia, and red cornflower, contains a *p*-hydroxy group on the phenyl ring. Cyanidin pigments of the red rose, blue cornflower, and deep red dahlia contain two phenolic hydroxy groups, and delphinidin (blue delphinium) has three hydroxy groups. Both mono- and diglycosides,



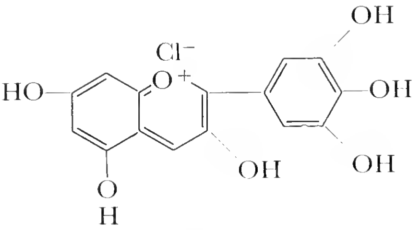
2-phenylbenzopyrylium chloride



pelargonidin



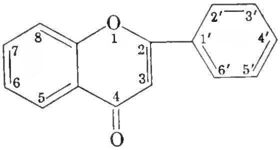
cyanidin



delphinidin

involving positions 5 and 7, are found in different species. Glucose is the most common sugar, although mixed diglycosides occur. Usually the alternate sugar is L-rhamnose.

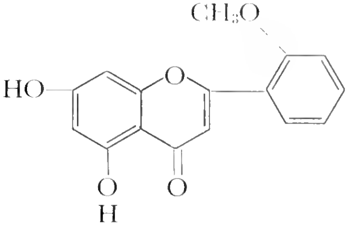
The colors of these pigments are sensitive to pH changes. For example, pelargonidin and cyanidin are red in acid solution and blue in an alkaline solution, whereas delphinidin follows a reverse pattern. In some species, such as the hydrangea, it is possible to control the color of the flower by adjusting the soil pH. Additional color variations are obtained by substituting methoxyl (CH_3—O—) for hydroxyl groups in positions 3′ and/or 5′, and sometimes in the 7 position. Hirsutidin, the coloring agent in blue grapes, is the 4-hydroxy-3′-5′-7-trimethoxy-2-phenylbenzopyrylium salt. Flavones, the water-soluble yellow and orange pigments, are derivatives of 2-phenylbenzopyrone.



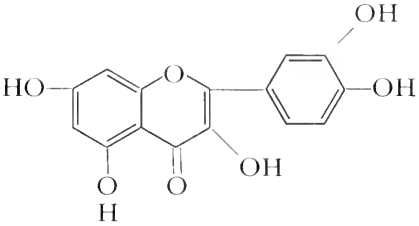
2-phenylbenzopyrone

Again, as in the anthocyanins, the members differ in the degree of hydroxylation. The flavonols have the basic structure of the flavones with the addition of a hydroxyl group in the 3 position. Generally flavones and flavonols carry hydroxyl groups in the 5 and 7 positions.

Citronetin, the coloring agent of the citrus peel, is shown as an example of the flavones. It is found as the glucoside. Quercetin, a component of oak bark and yellow coloring agent in corn plants, is illustrated as an example of the flavonol class. The flavonols have been reported to possess vitamin activity in the animal. Extracts of citrus peel and other sources of flavonols appear to supplement vitamin C in the prevention of capillary fragility. Rutin, a diglycoside of quercitrin, contains galactosylglucose and rhamnose and is isolated from buckwheat for medical purposes.



citronetin



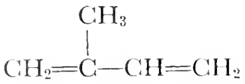
quercitin

The inheritance of the anthocyanins has been studied in a number of higher plants. Particular genes are known to cause the replacement of pelargonidin anthocyanins by the corresponding cyanidins. The change requires the addition of a hydroxyl group in the 3′ position of the basic nucleus. No biological function, other than possible attraction of insects for pollination purposes, has been ascribed to the water-soluble plant pigments.

Essential Oils

Essential oils have been so named because of their odor or essence. The fraction may be recovered from plant material by extraction with the usual fat solvents or by steam distillation. If the former method is used, distillation is necessary to separate the terpene derivatives from the phospholipides, carotenoids, and other components of the lipide fraction. Essential oils are members of a large group of compounds which may be considered as derivatives of isoprene, a 5 carbon diene.

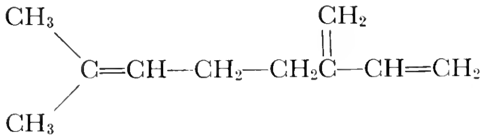
The group includes unsaturated hydrocarbons or monoterpenes ($C_{10}H_{18}$), sesquiterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes. The carotenoids are examples of tetraterpenes, and rubber and gutta-percha are examples of polyterpenes. Isoprene itself is not found in plant material.



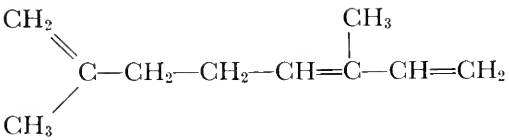
$$CH_2{=}\overset{\overset{\displaystyle CH_3}{|}}{C}{-}CH{=}CH_2$$

isoprene

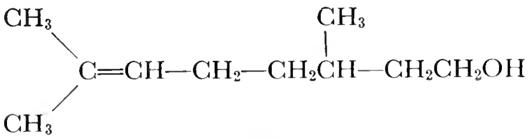
Essential oils are produced in quantity in only a small number of plants compared with the whole plant spectrum, 2,000 of about 400,000 total species. The oil is formed in special cells or in glandular hairs of the leaf and stem. In pine species the terpene and accompanying resin are formed by cells which line the resin canals of the bark. The oil is obtained by tapping these canals. Over 500 different chemical constituents have been identified in the essential oils, and these compounds have been classified on the basis of the number of isoprene equivalents per molecule. Monoterpenes (two isoprene equivalents) represent a large number of essential oil components. The simplest are the unsaturated acyclic hydrocarbons myrcene and ocimene. The well-known essential oils citronellol and geraniol are closely related to myrcene. Similarly, by elimination or shifting of a double bond or



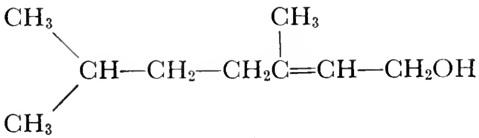
myrcene



ocimene

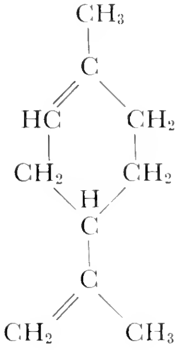


citronellol

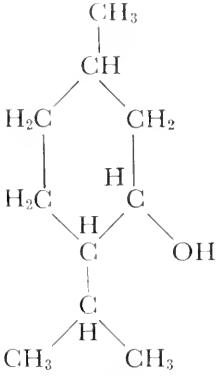


geraniol

by oxidation to the aldehyde, a whole group of acyclic monoterpenes is formed by the different plants. In other monoterpenes ring closure occurs to form substituted cyclohexenes or cyclohexadienes, for example, limonene.

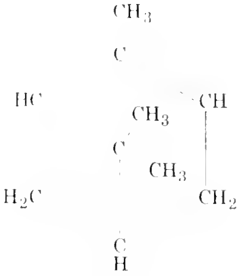


limonene

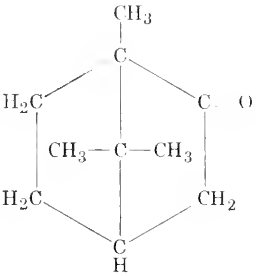


menthol

By oxidation at various positions in the ring or side chain related compounds like menthol are synthesized by various plants. Some species bring about a ring closure between the isopropyl side chain and the six-membered ring to form bicyclic structures. α-Pinene, the

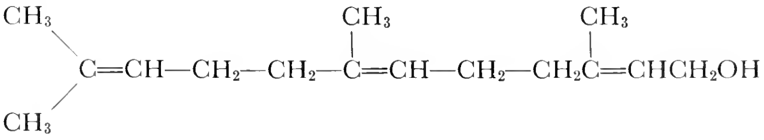


α-pinene



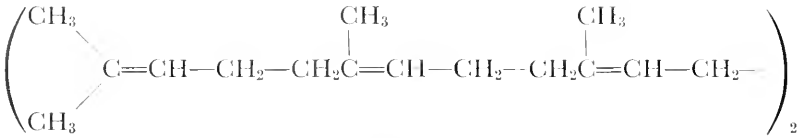
camphor

major component of the essential oil of a number of *Pinus* species, has such a structure. Camphor is an example of a bicyclic oxidized terpene. Sesquiterpenes contain three isoprene equivalents, with acyclic, cyclic, and polycyclic members reported from different species of plants. Rose oil and oil of citronella contain the acyclic sesquiterpene



$$\mathrm{(CH_3)_2C{=}CH{-}CH_2{-}CH_2{-}\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_2{-}CH_2\overset{CH_3}{\overset{|}{C}}{=}CHCH_2OH}$$

farnesol

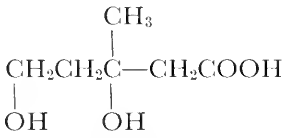


$$\left((CH_3)_2C{=}CH{-}CH_2{-}CH_2\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_2{-}CH_2\overset{CH_3}{\overset{|}{C}}{=}CH{-}CH_2{-}\right)_2$$

squalene

farnesol. The diterpenes occur infrequently in nature. One triterpene, squalene, found in higher plant oils, is presumably an intermediate in sterol synthesis.

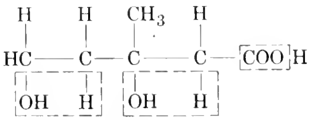
Although the terpenes are traditionally considered to be derivatives of isoprene, mevalonic acid has been implicated as an active intermediate in squalene synthesis. *In vitro* studies using purified enzyme fractions from rat liver have shown a rapid incorporation of mevalonic acid into squalene when incubated with manganese or magnesium ions, ATP, TPN+, and glucose-6-phosphate. The cofactors for synthesis using plant enzymes have not been examined in this detail.



$$\underset{OH}{\underset{|}{CH_2}}CH_2\underset{OH}{\underset{|}{\overset{CH_3}{\overset{|}{C}}}}{-}CH_2COOH$$

mevalonic acid

Probably the same metabolic intermediate functions in syntheses of mono-, di-, and polyterpenes. The relationship to the basic isoprene unit is evident, requiring only the removal of two molecules of water and decarboxylation.



$$H\overset{H}{\overset{|}{C}}{-}\overset{H}{\overset{|}{C}}{-}\overset{CH_3}{\overset{|}{C}}{-}\overset{H}{\overset{|}{C}}{-}\boxed{COO}H$$

The carotenoids (tetraterpenes) and other photochemical pigments are discussed in Chapter 9. These high-molecular-weight terpenes are non-volatile and therefore are not classified as essential oils.

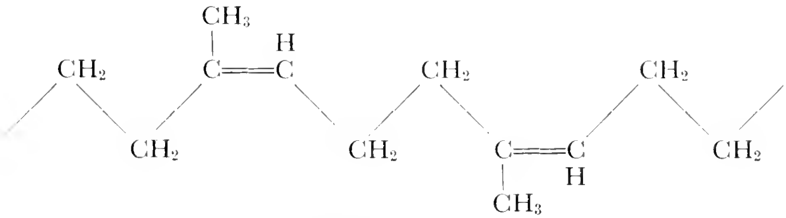
Plants contain a number of *volatile* or essential oils which are not members of the terpene class. Oil of wintergreen (methyl salicylcate) from the mint family, alloisothiocyanate of the Cruciferae, and anthranilic acid esters (components of the odor of orange blossoms) are examples of this varied group.

The essential oils have no known metabolic function in the plant. They may be of service to the plant through their attraction of insects, thereby aiding fertilization. Other members seem to repel insects as a result of their pungent odor.

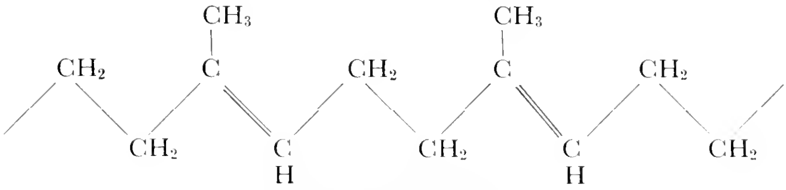
Rubber

Rubber and the related gutta-percha are representatives of the polyterpene class. Rubber has been detected in greater or lesser quantities in several thousand species of plants, although only a few of them contain sufficient quantities to be considered significant. The ability to produce rubber is scattered through a large number of plant families, but is not found in monocotyledonous plants, gymnosperms, or any of the lower plants. All the rubber producers are among tropical or temperate plants. Gutta-percha is produced by a few tropical species, especially by *Palaquium gutta* and *Mimusops balata*. *Achras sapota,* a tree from Central America, is the source of chicle, the base for chewing gum. Chicle is a mixture of gutta and triterpenols.

Structurally, rubber and gutta are isomers. The hydrocarbons of rubber possess the *cis* configuration, whereas the *trans* configuration predominates in gutta-percha. The latter also has a lower molecular weight than rubber. Molecular weights of 30,000 to 100,000 have been reported for gutta, whereas the molecular weight of rubber is 300,000 to 500,000.



central unit in rubber
cis configuration about double bonds



central unit in gutta-percha
trans configuration about double bonds

Rubber occurs in the plant in the form of microscopic particles suspended in the serum of specialized latex cells or vessels. Synthesis takes place in the cytoplasm of the cell. Rubber is suspended in both the cytoplasm as minute particles and the vacuolar fluid as larger particles. Recent reports have indicated that mevalonic acid (page 202) is the precursor in rubber synthesis, just as in the triterpene, squalene.

There is no known role for rubber in plant metabolism. The literature contains suggestions that rubber may be a reserve food, although the evidence available does not support such a view since plants subjected to prolonged starvation show no decrease in rubber content. Enzymes capable of attacking rubber are not known in higher plants but have been detected in microorganisms.

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Plant metabolism

9

All organic compounds found in plants result from the complex of reactions termed metabolism. These processes obviously must be exceedingly diverse to involve such a variety of substances. Although knowledge of the reactions occurring is increasing rapidly, there is still a vast amount to be learned. The complete story may never be written and probably cannot even be approached for many years. Any schemes drawn now must necessarily be tentative and subject to extensive revision as work progresses. Yet the available information is fundamental to an understanding of plant physiology and therefore should be summarized in any book on general biochemistry.

Fortunately, the metabolism of plants and animals is similar in many respects, making it unnecessary to cover all the reactions in detail for both kingdoms. The systems outlined below have not been demonstrated for any single species but represent a combination of the results from studies on a number of different plants. This treatment assumes that a reaction observed in two or more species is widespread and leaves the exceptions to subsequent work. Occasional errors have proved embarrassing, but this approach has been satisfactory in general.

The metabolism of any given compound bears either a direct or an indirect relationship to the metabolism of all the other compounds of the plant. For convenience, however, the metabolites are grouped into major classes. An attempt is made to indicate the interrelationships between these groups of compounds. In addition, special attention is devoted to photosynthesis and carbon dioxide fixation, processes rather characteristic of plants but not limited to them.

PHOTOSYNTHESIS

The prolonged existence of terrestrial life depends upon utilization of energy from the inanimate environment. It is believed that all species during the early periods of life on earth extracted energy from compounds made by purely geochemical processes during the preceding ages. Ultimately the substances containing useful quantities of chemical energy would be used up except for residues buried deeply or restricted to regions of prohibitively high or low temperatures. Life thus limited exclusively to chemical energy must necessarily cease or dwindle to a very low level compatible with the slow geological synthesis of substrates.

However, during the course of evolution another supply of energy came into use. Sunlight of the visible range was converted into chemical energy by new species carrying on a metabolic process called photosynthesis, named from the light-induced syntheses taking place. Modern photosynthetic plants require only simple inorganic materials and light. With the radiation absorbed they meet the fundamental requirement for energy needed in the syntheses of the organic compounds on which life depends.

All species capable of using carbon dioxide or bicarbonate ion as their sole source of carbon are called autotrophs. This group includes the ordinary green plants, algae, photosynthetic bacteria, and certain bacteria using chemical energy stored in inorganic compounds like hydrogen sulfide. Algae and the higher plants appear to employ the same or very similar mechanisms for photosynthesis, and the discussion here will be limited to this system.

All other living species require a continuing supply of from one to many organic substances. This requirement makes such forms (called heterotrophs) dependent upon photosynthesis for the energy stored in these organic compounds. The world is now essentially devoid of the original supply of foodstuffs produced geologically. The term *heterotrophs* includes the entire animal kingdom, most bacteria, and saprophytic (non-photosynthetic) plants like the fungi. Hence photosynthesis occupies a central position in the metabolism of plants and indirectly in that of animals.

Although photosynthesis leads to many products evolved by many reactions in the plant, the overall process may be indicated by

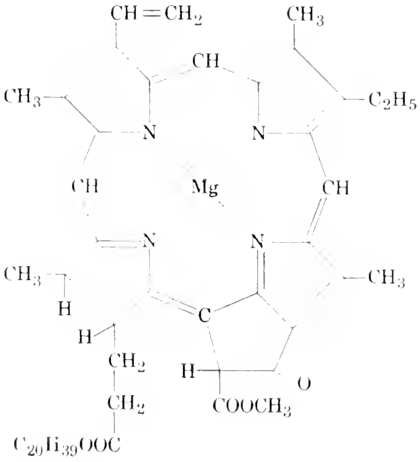
$$6CO_2 + 6H_2O \xrightarrow[\substack{\text{pigments}\\\text{enzymes}}]{\text{light}} \underset{\text{glucose}}{C_6H_{12}O_6} + 6O_2 \qquad \Delta F = +688{,}000 \text{ cal.}$$

where the free energy required comes from the light absorbed by the pigments, and the chemical reactions involved are catalyzed by enzymes. This reaction is the reverse of that shown on page 165 and considers only one of the multitude of actual products. For other products the quantities of energy, carbon dioxide, water, and oxygen vary in accord with the structure obtained.

Energy Absorption

The initial event in photosynthesis is the absorption of radiant energy. Apparently radiation of the long wave lengths of the infrared region is readily absorbed by plants as heat but is not sufficiently high in energy to induce the photosynthetic process. The available supply of ultraviolet light is limited and probably not much of a factor either. However, visible light is extensively absorbed by the specialized pigments mentioned in connection with the above overall reaction. All photosynthetic plants contain one or more of the group of pigments called chlorophylls.

Chlorophylls are structurally related to the porphyrins serving as prosthetic groups in hemoglobin, catalase, and the cytochromes (pages 469 and 151). Chlorophyll *a* seems to be of special importance and has the structure shown. In chlorophyll *b* the uppermost methyl group as written here is replaced by an aldehyde group. This latter pigment occurs with chlorophyll *a* in the higher plants. Other chlorophylls are associated with chlorophyll *a* in bacteria and some of the algae.

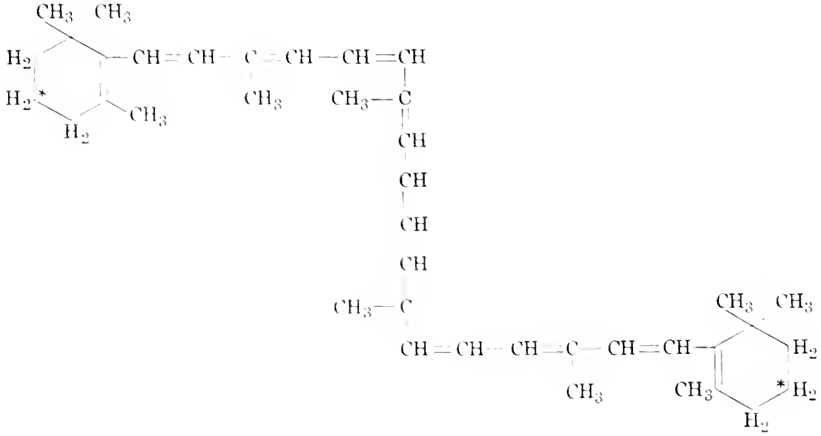


chlorophyll *a*

Chlorophyll *a* strongly absorbs light in the blue and red regions, and these wave lengths are effective in promoting photosynthesis. Green and yellow lights are absorbed by chlorophyll much less effectively, and reflection over this range leads to the characteristic color of the green plants. Some species are highly pigmented with other colors but nevertheless contain chlorophyll *a* masked by the additional pigments.

Chlorophyll *b* has its short wave length band shifted a little toward the blue-green. Thus it absorbs light not effectively absorbed by chlorophyll *a*. Moreover, the energy absorbed by chlorophyll *b* is transferred to chlorophyll *a*. Other photosynthetic pigments appear to function in a similar way. The carotenoids of higher plants absorb intermediate wave lengths, transferring at least part of the energy thus acquired to chlorophyll *a*. Still other types of pigments perform this function in the red, brown, and blue-green algae, providing for the utilization of part of the radiant energy that would otherwise be converted to heat and be of minor use in metabolic syntheses.

The widely distributed carotenoids are divided into two groups, carotenes and xanthophylls. Two of the most abundant representatives are β-carotene and lutein. Structural changes lead to other carotenes, and the possibility of *cis-trans* isomerism at many positions gives many more. A large number have been isolated. β-Carotene is



β-carotene

related to the vitamin A of importance to animals. Lutein has hydroxyl groups at each of the two positions marked with * in the structure of β-carotene. In addition, the double bond in one ring is shifted downward one position, corresponding to α-carotene. Other xanthophylls are also hydroxy or keto derivatives of various carotenes or ethers or esters of such compounds.

A strong absorption of visible light by organic compounds is associated with extensive conjugation (alternating single and double bonds) in the molecules. The intensity and wave length of the absorption are determined by the extent of the conjugation and the types of atoms involved, some producing a greater effect than others. As indicated by the structures for chlorophyll *a* and β-carotene, the photosynthetic pigments are highly conjugated, making them quite efficient for the absorption of light.

High efficiency is necessary to permit plant growth and even survival in rainy climates, in shaded locations, or during protracted cloudy weather. On the other hand, prolonged intense illumination may provide so much energy that utilization lags behind the supply. Serious injury could result unless some safety mechanism is provided. In plants photosynthesis begins at low light intensities and increases with the intensity to a limiting value (Figure 9–1). Absorption of light beyond the break in the curve seems to have little effect at moderate temperatures. Apparently the chlorophylls are protected somehow from photochemical destruction. One theory attributes a protective role to the carotenoids, but the problem is by no means solved. Photosynthesizing surfaces are normally at temperatures above that of the environment. When the external temperature rises much above 40°C., leaf temperatures in bright light rise still more, photosynthesis slows down or stops, and leaf damage or death of the plant may occur. However, these effects are attributed to excessive temperatures rather than an excess of visible light, since prolonged exposure to brilliant sunshine is not destructive at moderate environmental temperatures.

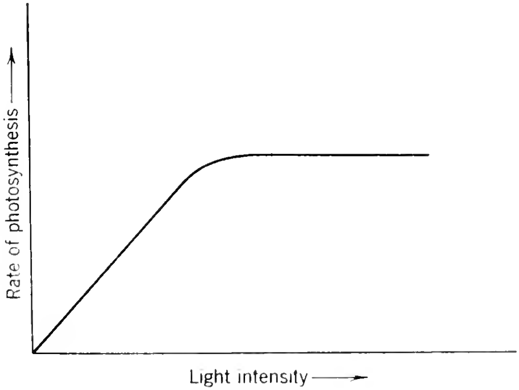


FIGURE 9–1. The effect of light intensity on the rate of photosynthesis.

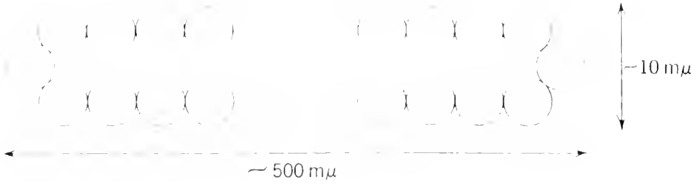


FIGURE 9–2. A diagram of a lamella (disc) of a granum of tulip chloroplast. Viewed from above instead of edgewise, the lamella is circular. Each circle in the diagram represents a spherical macromolecular component which is aligned with its neighbors to form the indicated flattened envelope. About ten of these lamellae are stacked vertically to form the granum. In the chloroplast itself, the grana are distributed throughout a matrix called the stroma.

Absorption of light by the photosynthetic pigments must result in structural changes of some sort in these pigments. It is now believed that these changes involve shifts of electrons to higher energy levels corresponding to excited states. Photosynthesis may require particular excited states reached only by absorption of light having definite energies. One current theory assigns two unpaired electrons to the excited molecule in contrast to the completely paired electrons in the systems of normal molecules. The energy inherent in the unpaired electrons comes from the absorbed light and is transferred along the series of pigments to chlorophyll *a*. Postulated mechanisms of transfer require a series of pigments placed relatively close to one another, suggesting an organized structure. (See Figure 9–2.) The mechanism of utilization of the energy of excited chlorophyll *a* is still largely unknown. Somehow the energy is used to dissociate water, leading ultimately to the formation of oxygen and the reduction of carbon dioxide.

The photochemically active structural unit anticipated above, called the chloroplast, is readily observed in and isolated from green plants. Its structural organization has not been resolved, but it is believed to contain the pigments and enzymes participating in photosynthesis. In fact, isolated chloroplasts supplied with suitable cofactors carry out some of the processes of photosynthesis.

Reaction of Water

During photosynthesis, the absorption of light and the transfer of the resulting energy from one pigment to another are followed by chemical reactions leading to all the components of cells. Many of these steps are only remotely concerned with photosynthesis and will be discussed later in the other sections on metabolism. The reactions directly related to photosynthesis can be grouped into two separate processes, the decomposition of water and the fixation and reduction of carbon dioxide. Such a division of the reactions can be achieved experimentally by illuminating green cells in the absence of carbon dioxide, then admitting carbon dioxide in darkness. In the first stage reducing material is formed photochemically from water, and this material reduces carbon dioxide in the second stage. The overall reaction of photosynthesis may thus be written in the form of a pair of consecutive reactions,

$$2H_2O + 2A \xrightarrow{\text{light}} 2AH_2 + O_2$$

$$CO_2 + 2AH_2 \xrightarrow{\text{dark}} (CH_2O) + 2A + H_2O$$

where A denotes the hydrogen acceptor system and (CH_2O) the reduced carbon.

Isotopic tracer experiments indicate that the oxygen evolved during photosynthesis probably comes from the water rather than directly from the carbon dioxide or organic materials. It is generally agreed that there must be more than two reactions in the formation of oxygen, but the sequence has not been worked out. Undoubtedly the water is somehow activated by energy absorbed in the pigment system. Excited molecules, free radicals, or perhaps some unknown special intermediates are formed and possess sufficient energy to transfer hydrogen to a suitable acceptor.

The natural hydrogen acceptor in the photolysis of water has not been identified, and perhaps different species employ different compounds. However, in the test tube, chloroplasts promote the transfer of hydrogen from water to any one of a variety of acceptors. The only known requirement in this isolated system concerns the oxidation-reduction potential of the acceptor used. Apparently the size and nature of the molecule are unimportant if the redox requirement is met. Measurements on the actual potentials in cells have been correlated with the properties of common biological systems. Of these, only two of the cytochromes (*f* and *c*) and ascorbic acid seem suitable, but proof is still lacking. In any case, the energy stored chemically in the hydrogen acceptor becomes available for the synthetic needs of the cell, including carbon dioxide fixation and reduction.

The energy requirement for the overall photosynthetic process as written

$$CO_2 + H_2O \rightarrow (CH_2O) + O_2 \qquad \Delta F = 115{,}000 \text{ cal.}$$

is much too high for transfer in any single biological reaction. This supposition also suggests the occurrence of a number of reactions with the energy derived from the light divided into suitable parcels. Complexity is normally associated with loss of efficiency. Hence there has been interest in learning how much of the light absorbed is converted to chemical energy and how much is lost as heat. Although higher efficiencies may be attained by cells under special conditions, 25 to 30 per cent seems to represent a more normal conversion of light to chemical energy. This value represents the ideal of conversion while the factor under field conditions is much smaller, in the neighborhood of 1 per cent. In the field much of the incident light is not absorbed by the plants and much is absorbed at intensities beyond the maximum rate of photosynthesis and is thus lost as heat. On cloudy days efficiency is higher than in full sun, but the total photosynthetic product of plants actually may be reduced because the lower sheltered leaves do not receive enough light to permit operation at full chemical capacity.

Fixation of Carbon Dioxide

There is more concrete information about the fixation and reduction of carbon dioxide than about any other aspect of photosynthesis. The overall reaction

$$CO_2 + 2AH_2 \rightarrow (CH_2O) + 2A + H_2O$$

is known to require several steps, and there are certainly others still unknown. The present conception of the reaction pathway coupled to photosynthesis is represented schematically in Figure 9–3.

Ribulose-1,5-diphosphate is believed to be the carbon dioxide acceptor and to go through the six-carbon acid to two molecules of 3-phosphoglycerate. The next step, transformation to 3-phosphoglyceraldehyde, requires energy in the form of TPNH. This high-energy compound and the ATP needed for the formation of the ribulose-1,5-diphosphate presumably arise from energy transfers following the absorption of light. Although the details of the relationship of ATP and TPNH to chlorophyll *a* are unknown, the energy from chlorophyll functions by way of these cofactors.

The role of the erythrose-4-phosphate is inferred from the other components of the reactions, but the actual participation of this particular compound has not been established in cells. Further work will probably alter this scheme somewhat by deleting or adding steps with the possibility of specific systems for different species. It is unlikely that the ultimate pathway will be simpler. Probably it will be more complex.

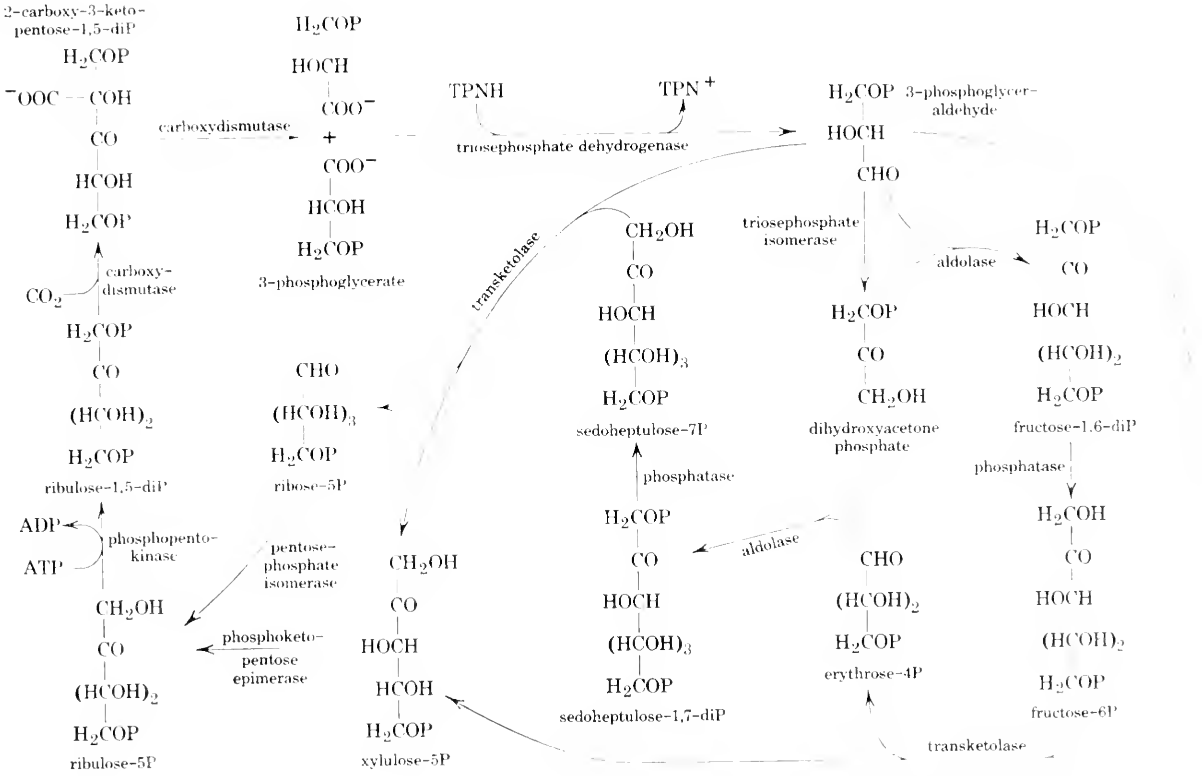


FIGURE 9-3. The path of carbon in the photosynthetic reduction of carbon dioxide as visualized in 1959. In simplifying the diagram the phosphate group is represented by the letter P in both structures and names, and straight-chain formulas have been written instead of the ring formulas to save space. Moreover, most of the cofactors have been omitted and the arrows pointed to indicate the direction of reaction in light. Actually many of these reactions are reversible.

Three clockwise trips around this cycle are needed to fix three molecules of carbon dioxide and yield a net gain of one molecule of 3-phosphoglyceraldehyde. Actually, of course, none of these intermediates accumulates extensively during photosynthesis. Instead the extra material is rapidly converted to other carbohydrates, especially sucrose and starch. Since reactions of 3-phosphoglyceraldehyde lead in so many different directions, the carbon dioxide fixed is rapidly distributed among several different intermediates.

Carbon dioxide is also fixed by reversal of some of the normal equilibrium reactions of respiration. The extent to which these processes operate in living cells is difficult to evaluate because ribulose-1,5-diphosphate fixes carbon dioxide even in the dark. This latter contribution is limited unless TPNH is available for the reduction; however, it does complicate the interpretation of tracer experiments, making the extent of fixation by other mechanisms uncertain. These secondary systems are indicated as reversible processes in the appropriate metabolic diagrams; for example, see pages 168 to 171.

CARBOHYDRATE METABOLISM

The carbohydrates of plants are all synthesized by the plants themselves and depend on reduction of carbon dioxide for the carbon needed. Light absorbed during photosynthesis serves by means of energy conversion and coupling mechanisms as the driving force in the synthesis of carbohydrates. Many of the individual reactions are markedly endergonic (requiring free energy). The energy needed comes primarily from the high-energy nucleotides as ATP, DPNH, and TPNH formed during the early stages of photosynthesis.

Plants put their carbohydrates to many uses, often as components of special substances like nucleic acids and glycosides. Other carbohydrates are structural materials. Probably little if any carbohydrate from these categories is ever reused by the plant under normal conditions. However, plants do respire in the dark and to a smaller extent in the winter during dormancy. This respiration may be the automatic result of the presence of enzymatic equipment and serve no useful purpose. More probably, respiration supplies energy needed for tissue repair and replacement of compounds like proteins that might otherwise break down spontaneously. Furthermore, plants definitely grow at night during the appropriate seasons and must depend on respiration for energy. This energy is stored chemically, of course, in the form of reserve materials, principally carbohydrates and lipides.

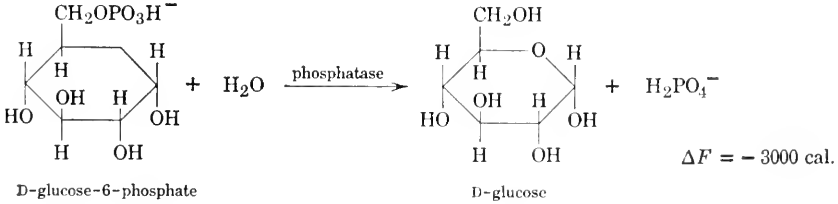
To a great extent the utilization of reserve carbohydrates is a reversal of the pathways of synthesis. Therefore, a separate discussion of these two processes is quite artificial. Nevertheless, for purposes of emphasizing the different goals concerned, such a division will be made here. Obviously many cross references will be necessary in avoiding extensive repetition.

Synthesis

Plants synthesize carbohydrates from carbon dioxide by fixing this substance and then reducing the product to carbohydrate derivatives. Some of the interconversions of these derivatives (phosphate esters) have been presented as part of the pathway of glycolysis (page 168) and as part of the system for the reduction of carbon dioxide during photosynthesis (page 213). Since the glycolytic reactions between glucose and 3-phosphoglycerate are known to be reversible, formation of the latter as a major intermediate in photosynthesis assures formation of glucose. All the intervening compounds thus become available for conversion to still other carbohydrate derivatives.

A consideration of the two figures referred to above reveals reactions for the formation of derivatives of the following monosaccharides: glycerose (glyceraldehyde), dihydroxyacetone, erythrose, xylulose, ribulose, ribose, fructose, glucose, and sedoheptulose. All these sugars belong to the D family except, of course, the optically inactive dihydroxyacetone. They are here listed in order of increasing size, two trioses, one tetrose, three pentoses, two hexoses, and one heptose. Only the last three of these occur in nature to any appreciable extent as the free monosaccharides, sedoheptulose being most abundant in succulent plants, for example, the *Sedum* group for which this sugar was named.

Free monosaccharides are formed from the corresponding phosphates when the ester linkage is split by enzymes called phosphatases. Although the substrate specificity of this group has not been clarified, the enzymes appear to be of great importance. Note the phosphatases at two steps in the scheme of photosynthetic reduction of carbon dioxide. The hydrolyses catalyzed yield inorganic phosphate and energy. They go rapidly and irreversibly so far as is known, as shown by the following:



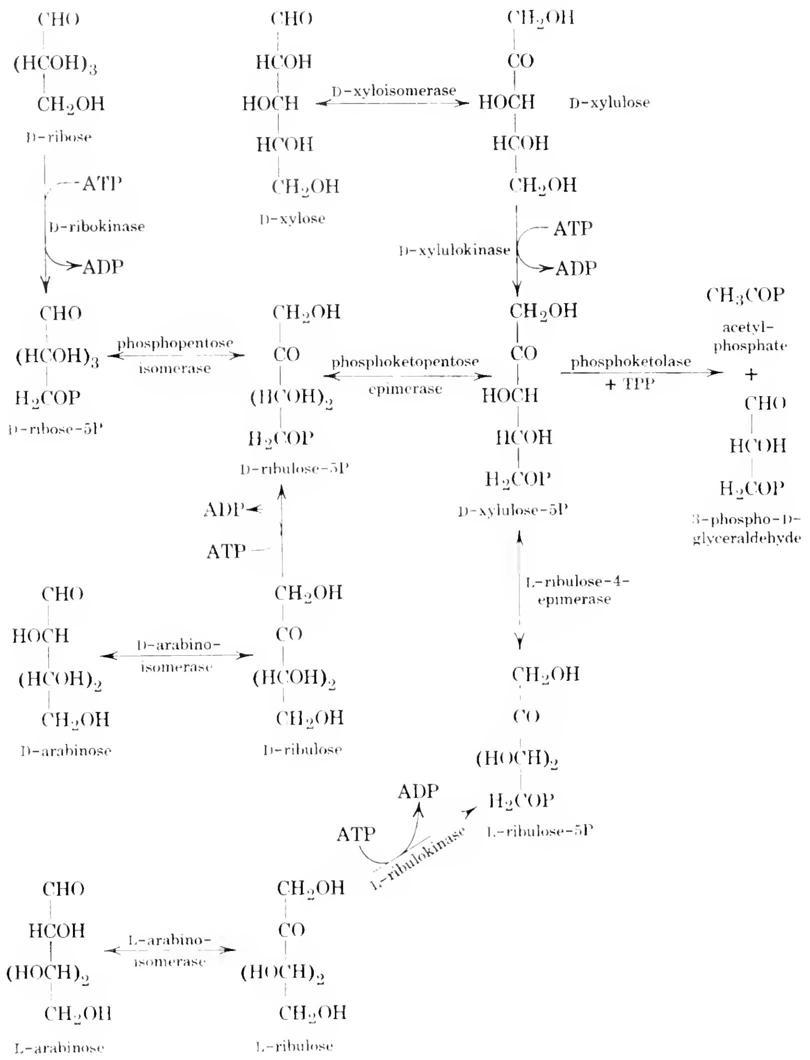


FIGURE 9–4. The interconversions of certain pentoses as represented in 1959. The isomerization of D-arabinose to D-ribulose and the subsequent phosphorylation have not been studied extensively in plants, and the names of the enzymes probably involved have been omitted to indicate this deficiency. TPP signifies the cofactor thiamine pyrophosphate known to participate in other cleavage reactions of this type.

There may or may not be phosphatases in plant cells capable of attacking all the phosphate esters of the smaller monosaccharides mentioned thus far. But as yet there is little evidence of their role in nature except in the formation of glucose, fructose, and sedoheptulose.

Many other monosaccharides occur as plant components, and their biological syntheses are under investigation. Starting with the pentoses, some of the interrelationships are summarized in Figure 9–4. Note particularly the reaction connecting the D and L families, both of natural importance in the case of pentoses. Any small amounts of free pentoses are probably formed by the action of phosphatases rather than by reversal of the kinase-ATP reactions. The latter seem to be irreversible in known cases of this kind, but phosphatase action provides a potential mechanism for removal of the phosphate group and the formation of the pentoses themselves. Isomerases then convert the free ketopentoses into the related aldopentoses. This scheme provides for many of the pentoses, although some isomers are missing and lyxose is not considered. Lyxose does occur naturally in heart muscle, but little is known of its biochemistry. It differs from xylose in having the opposite configuration about the second carbon atom.

The compound central to the formation of many of the pentoses is xylulose-5-phosphate, which may itself be formed subsequent to the photosynthetic fixation of carbon dioxide (see Figure 9–3). Some mechanism may exist also for combining 3-phosphoglyceraldehyde with acetyl coenzyme A or the like to form xylulose-5-phosphate, perhaps by simple reversal of the right-hand reaction of Figure 9–4. Finally, this important intermediate in pentose synthesis probably appears during the metabolism of hexoses by way of the pentose-phosphate system (see Figure 9–6).

As indicated on page 213, 3-phosphoglyceraldehyde and dihydroxyacetonephosphate arise during the photosynthetic fixation of carbon dioxide. The two substances ultimately lead to glucose, fructose, mannose, and galactose as shown in Figure 9–5. The reactions converting fructose-6-phosphate to glucose-6-phosphate and glucose-1-phosphate are the reverse of those indicated on page 168 as equilibria. The reaction yielding galactose-1-phosphate is complex and has been represented by the step shown on page 219.

Thus the glucosyl and galactosyl residues are exchanged by this reaction and galactose-1-phosphate can be synthesized. Moreover, the enzyme system transforms uridine diphosphoglucose into urdine diphosphogalactose. Hence, if the two glucose derivatives are formed,

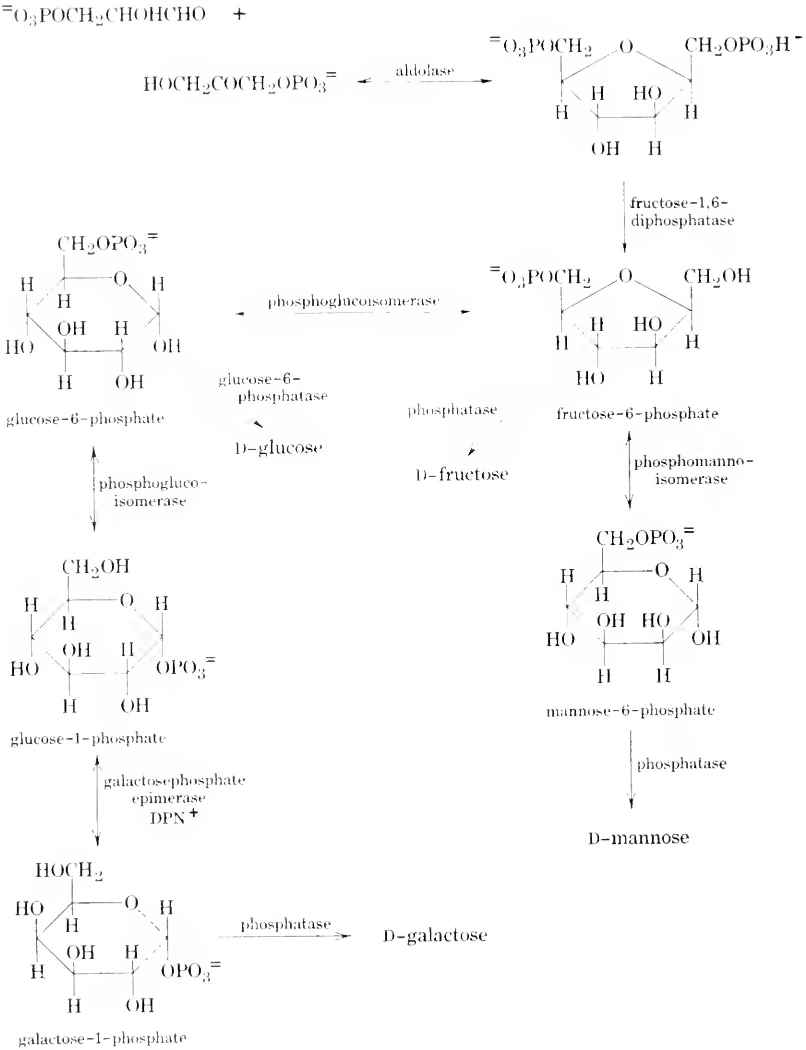
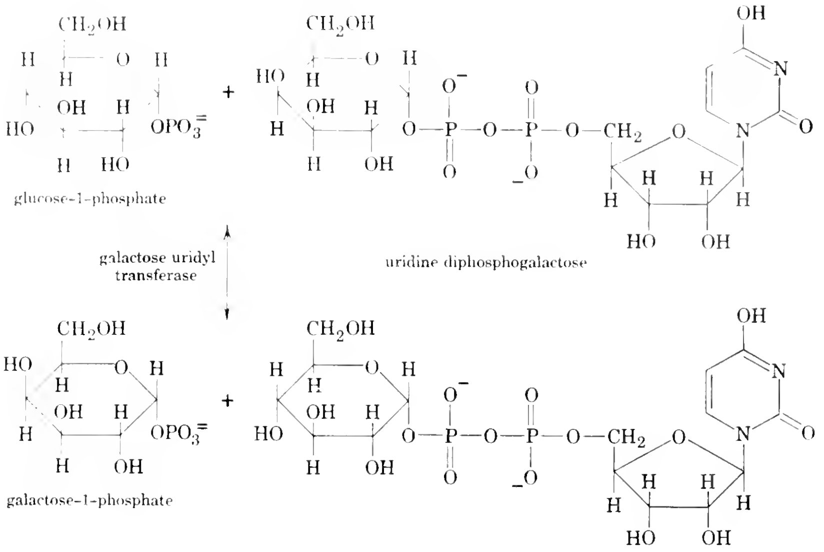


FIGURE 9–5. The formation of four hexoses as known in 1959. The reverse of these processes is presented in Figure 7–1. A requirement for DPN+ has been demonstrated in the galactosephosphate epimerase step, but the role played is unknown. Apparently the DPN+ is not functioning in its normal role of hydrogen-electron acceptor.



glucose-1-phosphate

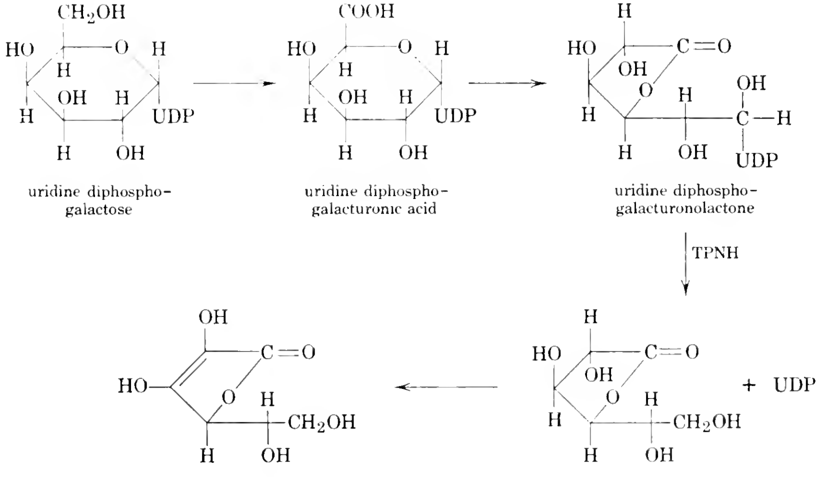
uridine diphosphogalactose

galactose-1-phosphate

uridine diphosphoglucose

galactose-1-phosphate appears as long as the equilibrium conditions are not satisfied.

The biological synthesis of L-ascorbic acid (vitamin C) may be taken as an example of the formation of special materials from hexoses. Studies with peas and mung beans indicate that this acid may be derived as follows, where UDP represents the uridine diphosphate portion of the molecule:



uridine diphospho-galactose

uridine diphospho-galacturonic acid

uridine diphospho-galacturonolactone

galacturonolactone

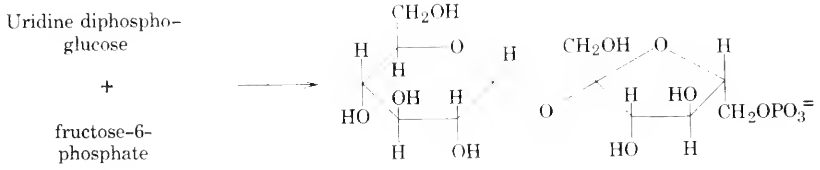
L-ascorbic acid

Work with strawberries suggests that an entirely different mechanism may be operating, so a need for further work is obvious.

Sucrose is one of the major end products of photosynthesis and for this reason is chosen as illustrating the formation of disaccharides. Two mechanisms are believed to operate, at least under some conditions.

$$\text{Uridine diphosphoglucose} + \text{fructose} \longrightarrow \text{sucrose}$$

or



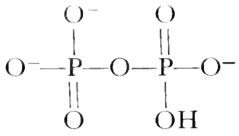
sucrose-6′-phosphate

$$\text{Sucrose-6}'\text{-phosphate} \longrightarrow \text{sucrose} + HOPO_3^{=}$$

The uridine diphosphoglucose consumed in these reactions and that of page 219 is thought to form according to

$$\text{Uridine diphosphate} + \text{ATP} \rightarrow \underset{\text{(UTP)}}{\text{uridine triphosphate}} + \text{ADP}$$

$$\text{UTP} + \text{Glucose-1-phosphate} \rightarrow$$

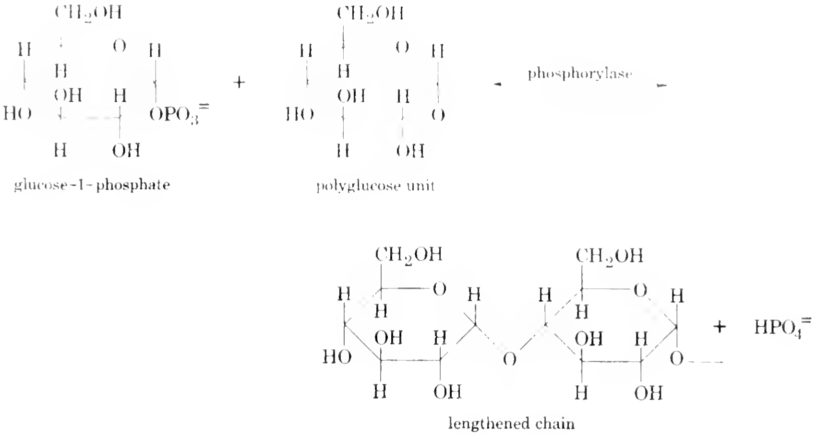


$$\text{uridine diphosphoglucose} + O^{-}\!-\!\underset{\underset{\textstyle O}{\|}}{\overset{\overset{\textstyle O^{-}}{|}}{P}}\!-\!O\!-\!\underset{\underset{\textstyle OH}{|}}{\overset{\overset{\textstyle O}{\|}}{P}}\!-\!O^{-}$$

pyrophosphate

In this way the uridine diphosphoglucose is made available for further reaction at the expense of ATP.

As indicated in the scheme for glycolysis (page 168), the polysaccharides are formed from hexosephosphates. Phosphorylase catalyzes the attachment of glucosyl groups to polyglucose units. These latter materials appear necessary as starters since the enzyme does not form maltose. The energy needed for the acetal bond comes from the glucosephosphate bond. This process continues until the molecules grow into amylose. Amylopectin, the other component of starch, results from the action of 4,6-transglucosidase, which shifts some of the glucose units on the ends from position 4 to 6 and forms the branches.



The mannose-6-phosphate discussed above is converted to mannose-1-phosphate by a mechanism analogous to that of pages 168 to 170 and for glucose-6-phosphate. Mannose-1-phosphate is probably used in synthesizing the mannans of plants by a mechanism similar to that for starch.

Glucose-1-phosphate is believed to serve in the synthesis of cellulose, the most abundant carbohydrate. Since the acetal linkage is β rather than α as in starch, another enzyme system than phosphorylase would be expected and has been isolated from bacteria.

Utilization

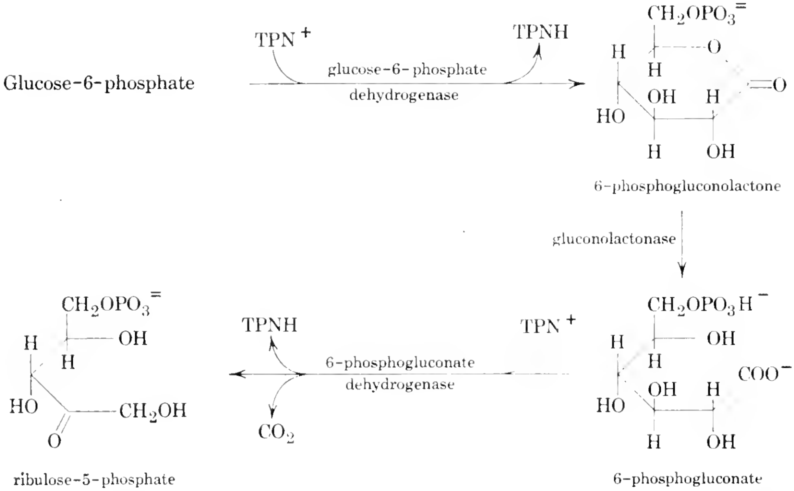
Plants respire in the dark and during all other periods when photosynthesis is not occurring, except perhaps at subfreezing temperatures when the multitude of reactions requiring water might be stopped or greatly retarded. In fact, many investigators feel that plants respire even during photosynthesis.

Much of this respiration utilizes carbohydrates as substrates including sugars, their derivatives, and polysaccharide reserves. Cellulose seems to be solely a structural substance as far as plants are concerned and is not metabolized once formed. The hemicelluloses of some plants may be metabolized under some conditions, but the primary cleavage reactions are unknown. Starches serve as the principal reserve carbohydrates and are converted by means of the 4,6-transglucosidase and phosphorylase to glucose-1-phosphate.

Plants probably metabolize free sugars of various kinds to some extent. But the comparative importance of mono- and disaccharides in the energy supply is unknown. Therefore, discussion of the mechanism by which the individual sugars are attacked is delayed, pending consideration of the metabolism of carbohydrates by animals, where important quantities of energy are known to be involved.

When formed from any source, part of the glucose-1-phosphate undergoes the reactions of the glycolytic or Embden-Meyerhof pathway diagrammed on page 168, leading to pyruvate. The pyruvate is then converted to carbon dioxide and water via the tricarboxylic acid cycle shown on page 171. In the higher plants and animals most of the energy obtained during respiration comes from the oxidations of the DPNH and TPNH formed during the degradation of pyruvate.

Not all the glucose-6-phosphate formed follows the Embden-Meyerhof-tricarboxylic acid cycle sequence. Most, perhaps all, higher plants, including pea, cucumber, and many seeds, convert part of this hexosephosphate to carbon dioxide by a different route. On reaching ribulose-5-phosphate, the system must involve several of the reactions written for the photosynthetic fixation of carbon dioxide. Although unsettled, the pentosephosphate pathway may be represented tentatively as in Figure 9–6.



As a central compound, the 3-phosphoglyceraldehyde may be metabolized by way of pyruvate and the tricarboxylic acid cycle. Or 3-phosphoglyceraldehyde and dihydroxyacetonephosphate may be joined by the adolase to form fructose-1,6-diphosphate. This last compound is readily converted to fructose-6-phosphate by a phosphatase, and the latter substrate converted to glucose-6-phosphate for another turn around the pentosephosphate pathway.

This latter arrangement eliminates one molecule of carbon dioxide for each turn around and provides two molecules of reduced coenzyme

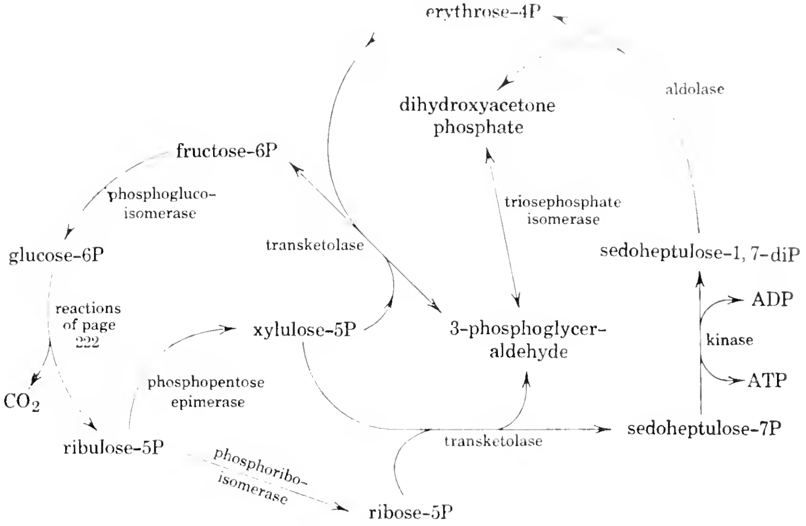


FIGURE 9–6. An outline (1959) of the transformations of the pentosephosphate pathway. Except for perhaps the decarboxylation step, these reactions are probably reversible. The kinase reaction is not reversible of itself, but a phosphatase can convert sedoheptulose-1,7-diphosphate to sedoheptulose-7-phosphate. Since ADP is not changed to ATP, this stage is not reversible in a strict sense.

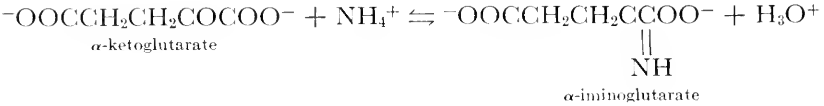
(TPNH). Hence the energy yield is probably similar by both metabolic systems. Undoubtedly both are used, but their relative importances are unknown. Obviously the metabolism of pentoses must at least start with the pentosephosphate pathway.

METABOLISM OF NITROGEN COMPOUNDS

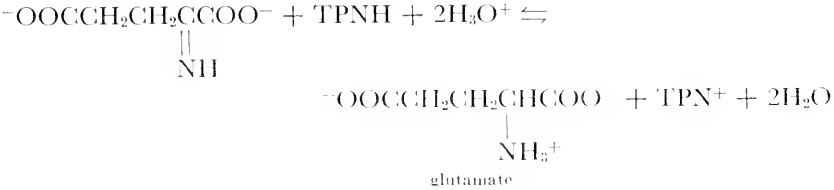
Much less is known about the metabolism of nitrogen compounds in plants than about that of carbohydrates. It is not yet possible to write general reaction patterns with any degree of certainty. Nevertheless, it is possible to discuss certain aspects of nitrogen metabolism from a broad point of view. Solely for convenience in this treatment the subject matter is divided into sections on inorganic and organic nitrogen, respectively.

Inorganic Nitrogen

The early atmosphere of the earth is believed to have been rich in ammonia. Species then probably depended upon the utilization of ammonium ion supplied by solution of this gas. It is probable that ammonium ion entered the metabolic systems of the organisms concerned by reaction with certain keto acids to form corresponding amino acids. This reversible process is now known to occur in a great variety of plants, animals, and microorganisms and may be practically universal.



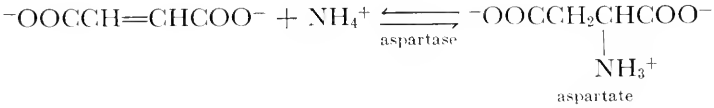
$$\underset{\alpha\text{-ketoglutarate}}{{}^{-}OOCCH_2CH_2COCOO^{-}} + NH_4^{+} \rightleftharpoons \underset{\alpha\text{-iminoglutarate}}{{}^{-}OOCCH_2CH_2\underset{\underset{NH}{\|}}{C}COO^{-}} + H_3O^{+}$$



$$\begin{aligned} &{}^{-}OOCCH_2CH_2\underset{\underset{NH}{\|}}{C}COO^{-} + TPNH + 2H_3O^{+} \rightleftharpoons \\ &\qquad \underset{\text{glutamate}}{{}^{-}OOCCH_2CH_2\underset{\underset{NH_3^{+}}{|}}{C}HCOO^{-}} + TPN^{+} + 2H_2O \end{aligned}$$

A large proportion of the glutamate required by plants is formed in this way. Indeed, this process is felt to be the central one in the utilization of ammonia. The α-ketoglutarate required is readily available from the tricarboxylic cycle, where it is formed during the normal metabolism of carbohydrates and lipides. The first step may be spontaneous, the second requires an enzyme called glutamic dehydrogenase, and the reduced coenzyme must be made available by means of energy from either photosynthesis or respiration.

Another mechanism for the incorporation of ammonia is represented as



$$^{-}OOCCH{=}CHCOO^{-} + NH_4^{+} \underset{\text{aspartase}}{\rightleftharpoons} \underset{\text{aspartate}}{{}^{-}OOCCH_2\underset{\underset{NH_3^{+}}{|}}{C}HCOO^{-}}$$

wherein this second amino acid is formed directly from inorganic nitrogen, employing still another intermediate from the tricarboxylic acid cycle. These two systems probably account for the principal consumption of ammonium ion.

Conversely they may also participate in an important way in the formation of ammonia during the decay of animal and vegetable matter. The organisms of decay convert amino acids into corresponding acids and ammonium ion. The organic compounds are metabolized, supplying the energy needed for the growth and reproduction of the organisms of decomposition. At the same time, some of the ammonia may be used, the rest set free for other species. Probably deaminations of a variety of types play natural roles in addition to the reversals of the reactions noted above. Most of the known cases involve microorganisms rather than ordinary plants and animals with one major exception, urea. Urea is a major product of animal metabolism and is undoubtedly widespread elsewhere. It is attacked in solution by a common enzyme according to

$$H_2NCONH_2 + H_2O \xrightarrow[\text{urease}]{} 2NH_3 + CO_2$$

As oxygen began to appear in the atmosphere during the evolution of the plant, microorganisms appeared which could obtain the energy they needed by the oxidation of ammonium ion. The mechanism of this reaction is not known but in *Nitrosomonas* may be written in the overall form

$$NH_4^+ + \tfrac{3}{2}O_2 + H_2O \rightarrow NO_2^- + 2H_3O^+$$

and is known to occur in soils, especially in neutral or alkaline soils as might be predicted from the appearance of hydrogen ions. Once nitrite ion is formed, another group of bacteria called *Nitrobacter* complete the oxidation.

$$NO_2^- + \tfrac{1}{2}O_2 \rightarrow NO_3^-$$

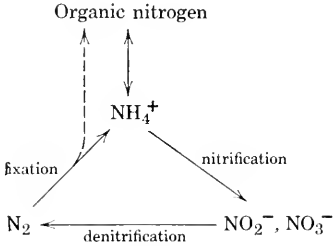
Neither nitrite nor nitrate is especially useful for plants and animals in this form, although plants probably reduce appreciable quantities of nitrate and utilize the products. Both ions are somewhat toxic to animals, and nitrite is especially dangerous even to plants. Under certain abnormal environmental conditions, large enough concentrations of potassium nitrate accumulate in oat straw, for example, to be fatal to livestock. Sometimes local concentrations of nitrites in poorly located farm wells have reached levels toxic to children.

Therefore, if ammonia were converted irreversibly to nitrate, the supply of available nitrogen would tend to dwindle. However, other bacteria called denitrifiers have evolved and reduce nitrate to nitrite and on to molecular nitrogen or to ammonia. These soil organisms must, of course, employ energy from natural reducing substances in the process. The inorganic compounds involved may include free sulfur, sulfide, thiosulfate, hydrogen. Various organic materials may serve, but there is usually some species specificity. Ordinarily the denitrification occurs in the absence or near absence of oxygen, and the nitrate and nitrite ions function as the oxidizing agents.

This process introduces a new complication, for in spite of scattered reports to the contrary, animals and most plants cannot employ molecular nitrogen in any of their metabolic processes. Hence, denitrification, mainly to nitrogen, puts this critical element into an unavailable form in the atmosphere. The evolutionary solution of this problem has taken the form of species fixing molecular nitrogen. Again energy is needed for the reduction, which is carried out aerobically by species of *Azotobacter*, anaerobically by *Clostridia*, and photosynthetically by the various photosynthetic bacteria, the blue-green algae, and members of the bacterial genus *Rhizobium* in collaboration with leguminous plants. The first two groups are bacterial genera and use organic energy sources. The last groups employ solar energy in reducing the nitrogen.

Knowledge of the mechanism of biological nitrogen fixation is of great potential importance to industry, biology, and agriculture. Yet in spite of a great expenditure of effort this mechanism is almost completely unknown. Many schemes have been postulated on the basis of indirect evidence of diverse kinds. Most of these schemes list inorganic compounds as intermediates between molecular nitrogen and ammonia. However, there is room for doubt that even the ammonia observed is a primary biological product. The present authors feel that the ammonia may be a by-product derived from one or more reactions of the types mentioned above in connection with the formation of ammonia from amino acids. It seems more probable that molecular nitrogen is fixed almost immediately in an organic form which is then reduced stepwise to amino nitrogen. Proof of this or any other theory appears to lie in the future.

These transformations may be summarized in the following nitrogen cycle:



The combination of *Rhizobium* and legume is agriculturally important in nitrogen fixation. This sort of association is designated as symbiotic, meaning that both biological components are essential for the process. In fact one of the major frustrations in the study of nitrogen fixation has been the lack of an experimental technique for obtaining fixation in cultures of these bacteria grown without plants. Under natural conditions the bacteria live in nodules on the roots of plants of the alfalfa, clover, pea, and bean groups. The plants supply something to the fixing system that has not been successfully replaced by plant extracts, metabolic intermediates, coenzymes, metal ions, or biological energy sources like ATP. The plant itself, of course, fixes no nitrogen. Ultimately, it will be possible to replace the plant with suitable chemicals, and the accomplishment will contribute greatly to an understanding of nitrogen fixation in general.

The importance of nitrogen fixation and denitrification is illustrated schematically in Figure 9–7. In view of the problems of feeding ever-increasing human populations, it is important that the available nitrogen of the soil be kept in balance to provide for the growth of our plant and animal foods. Indeed, the rapidly increasing number of human beings demands that soil nitrogen be increased to provide an adequate food supply. Likewise an increased use of the sea is indicated.

Unfortunately many people feel that losses of nitrogen from the land surface by nitrification and denitrification, leaching and erosion, and sewage exceed the gains from marine fertilizers (a limited factor at present) and fixation. If so, then the reservoirs are being depleted, especially in Asia, where the demands are greatest and biological fixation is the only significant means of replacement. In Europe and North America chemical fixation of nitrogen for fertilizers is becoming quite significant to the overall nitrogen supply of the soil. The United States in 1956 used chemical fertilizers, principally in the form of ammonia, ammonium nitrate and sulfate, calcium and sodium nitrates,

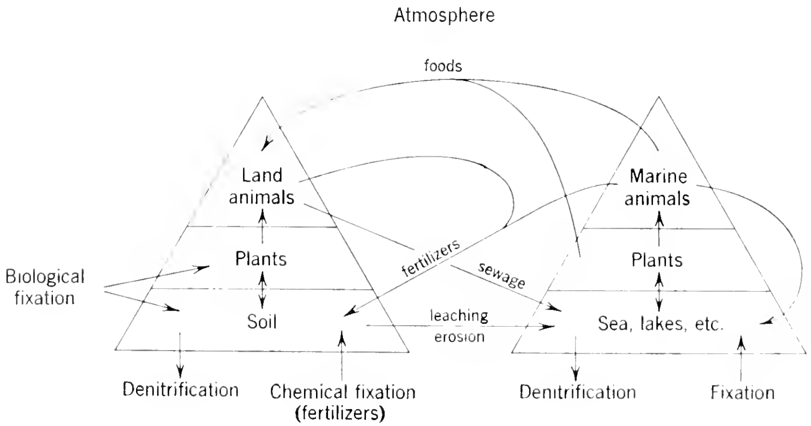


FIGURE 9–7. Some of the large-scale transfers and reservoirs of nitrogen. The critical role of nitrogen fixation in the maintenance of life is evident.

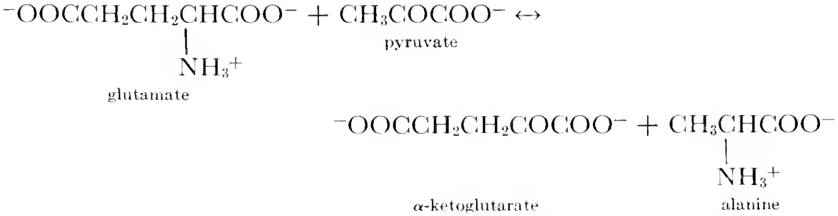
calcium cyanamide, and urea to the extent of approximately 2 times 10^6 tons of nitrogen.

Estimates of the biological fixation of nitrogen in the United States range from about 10^6 to 10^7 tons per year. In either case chemical fertilizers are becoming very significant to crop production in this country, especially since fertilizers are applied intensively to the crop lands, whereas biological fixation is distributed over the land surface as a whole. Even so, there is probably still a nationwide net loss of nitrogen from the agricultural areas, although this loss may be declining. Other countries are probably less fortunate. Biological nitrogen fixation on land may be about evenly divided between the symbiotic and non-symbiotic processes as occurring in the cultivated legumes bearing *Rhizobia* and in the *Azotobacter* and *Clostridia*, respectively.

Organic Nitrogen

The metabolism of organic compounds of nitrogen has been studied intensively in the last decade and much has been learned, particularly concerning the metabolism of amino acids, purines, and pyrimidines. Since higher plants and animals seem to employ similar pathways, only one discussion at the biochemical level is really necessary. Therefore, the pertinent details are assembled in Chapter 20 on nitrogen metabolism, in the section on the animal. It so happens that most of the work has been done with animals and microorganisms.

As mentioned earlier, when ammonium ion is absorbed by root cells much of the nitrogen appears first in glutamic and aspartic acids. The nitrogen thus accumulated is transported throughout the plant mainly but not entirely in the form of asparagine and glutamine, which probably are synthesized from the corresponding amino acids. Next, all cells seem to transfer the amino groups to appropriate α-keto acids, forming other amino acids as in the example below. The enzyme functioning is called a transaminase and requires a coenzyme called pyridoxal phosphate, discussed on page 340 under vitamin B_6. Glutamate ion or glutamic acid itself may be the amino donor in all cases, serving as a central intermediate. Since transaminations



$$\underset{\text{glutamate}}{^{-}OOCCH_2CH_2\underset{\underset{NH_3^+}{|}}{C}HCOO^-} + \underset{\text{pyruvate}}{CH_3COCOO^-} \leftrightarrow \underset{\alpha\text{-ketoglutarate}}{^{-}OOCCH_2CH_2COCOO^-} + \underset{\text{alanine}}{CH_3\underset{\underset{NH_3^+}{|}}{C}HCOO^-}$$

are reversible, various amino acids can be converted by such reactions into keto acids for respiratory metabolism. Probably not all amino acids are formed or decomposed directly by this process, but many are, including glycine, alanine, leucine, isoleucine, tyrosine, and perhaps others. Once these amino acids have been synthesized by transaminations, others are made from them. Starting with one or more of these substances, most of the other nitrogen compounds of the cells are made (see Chapter 20).

METABOLISM OF LIPIDES

Once again more work has been done with animals than with plants. However, some general features may be presented for plants. Triglycerides accumulate in higher plants, where they appear to serve as energy reserves, especially in seeds. Since little is known of the reactions involved in the utilization of fats by germinating seeds, attention will be limited here to the biosynthesis of fats in plants.

All the tissues of plants contain fats, but the amounts are small in most species except in the fruits and especially the seeds. Long ago it was believed that fats, or perhaps the component parts, were synthesized in the leaves and growing parts of plants and translocated to the fruits. Later, however, it was found that fruits detached from the plant could still make fats and that most if not all of the fat of the fruit was synthesized therein. As a result, studies of the biosynthesis of fats in plants have involved fruits and seeds.

Present opinion holds that the carbon of fats comes from carbohydrate. Radioactive carbon from glucose appears in the oil of seeds from maturing cotton bolls and in that from castor beans. It is logical to assume that any substance whose metabolism is connected to that of carbohydrates could supply carbon to the fats. This logical possibility must depend upon the occurrence of intermediates that can be transported to the fruit. Amino acids can be converted in some cases to keto acids of the tricarboxylic acid cycle and thus become available for synthesis. The branched-chain amino acids actually supply part of the carbon skeletons for branched-chain fatty acids now known to occur in small amounts. These and related speculations await examination.

Assuming that carbohydrates or their breakdown products are transported from leaves to fruits and converted to fats, the mechanism of this conversion becomes of interest for several reasons. First, the seeds of different species are specific in the kinds of fatty acids formed.

Oleic and linoleic acids together total about 80 per cent of the fatty acids of the seed fats of the world. Palmitic acid, next most abundant, may amount to 10 per cent, and all the others combined make up the remaining 10 per cent. Obviously the biosynthesis of these three most common acids is of first importance. Yet some seeds are extremely high in other fatty acids. For example, nutmegs produce 75 to 80 per cent of their fatty acids as myristic acid, palm species 50 per cent lauric and 20 per cent myristic acids, and seeds of Cruciferae 30 to 40 per cent erucic acid; in castor beans ricinoleic acid reaches 90 per cent of the total fatty acids. Thus the fats are to a large extent characteristic of the species concerned. Perhaps the various species possess the same or quite similar mechanisms for their biosynthesis, but if so they must control these mechanisms in quantitatively different ways to form such diverse products.

Carrying this matter of differences a step farther, there is evidence that flax, among other plants, forms oils of varying composition depending upon climatic location. The same major fatty acids are probably always present, but their proportions change. Moreover, it is clear that the proportions of fatty acids change during the maturing of fruits and seeds, indicating a change in the control process during ripening. Cottonseed, 31 days after flowering, contains 2.4 per cent lipides with 23.9 per cent saturated, 29.3 per cent oleic, and 46.8 per cent linoleic acids. After 29 days longer the total lipides reach 25 per cent with 22.4 per cent saturated, 25.5 per cent oleic, and 52.1 per cent linoleic acids. Such changes are probably typical and are much exceeded in soybeans and flaxseed.

A decrease in the percentage of components was taken for a time to mean that the fatty acids showing the decreasing percentages were being converted into those of increased percentage. It turns out that the saturated acids and oleic acid are relatively abundant in young seeds and decrease proportionately in the mature forms. This observation led to the theory that the saturated fatty acids are synthesized first and later dehydrogenated. Now, however, the discovery that all appear to increase in absolute amount, though at different rates during ripening of the seed, suggests that the saturated fatty acids are not precursors of unsaturated types. Instead different routes of synthesis have been suggested with changes in the rates with age to account for the observed changes in the proportions of fatty acids.

Castor beans and flaxseed utilize radioactive acetate in the synthesis of saturated fatty acids. The mechanism is speculated to be like the multiple condensation of acyl coenzyme A outlined on page 458 for animals. Such a scheme would account for the minor quantities of other fatty acids normally found with even numbers of carbon atoms. It does not explain why the other known acids of this series are not always found also, nor why palmitic or some other member is especially predominant. However, labeling experiments with either carbon atom one or two marked in separate studies lead to palmitic acid labeled only in the odd or even position in the two respective cases. This specificity of positioning strongly indicates an acetyl coenzyme A mechanism.

There is still difference of opinion about the biosynthesis of the unsaturated fatty acids. Some tracer experiments with acetate have led to radioactive oleic, linoleic, and linolenic acids in flax, but the radioactive atoms were not located to exclude other types of reactions. Studies with other plants failed to detect radioactivity in unsaturated fatty acids when labeled acetate was supplied. Thus another mechanism has been assumed and some have been sketched, but the problem is still quite open.

Traces of free fatty acids appear to occur with the fats themselves. Therefore, most investigators feel that the fatty acids are formed first and then are esterified with glycerol to complete the process. However, if the acyl coenzyme A route participates in fatty acid synthesis, the high free energy inherent in the fatty acid coenzyme A complex could be most effective in the esterification step. In this event the free acids would not participate as intermediates in fat synthesis but would play other roles.

Be that as it may, plants show some degree of specificity in the fats formed. In other words, the esterification step is not a hit or miss proposition, and the fats tend to be of certain specific kinds. When more than one fatty acid occurs in the fat in quantity, the triglycerides seem to be mixed rather than solely of the tripalmitin or triolein type. Furthermore, there is frequently a distinct specificity in the isomer produced. Cacao butter seems to contain β-oleodistearin, β-palmito-oleostearin, and β-palmitodiolein to the exclusion of the corresponding possible isomers. The last of these substances is quite widespread and occurs in quantity in olive and cottonseed oils, again only in the β form. However, other plants seem to contain both isomers of some triglycerides. Palm oil contains roughly equal quantities of the α- and β-oleodipalmitins, but at least 90 per cent of the palmitodiolein is the unsymmetrical isomer.

This rather marked selectivity, though certainly not universal, obviously requires some sort of biosynthetic control, imposing another condition on the mechanism. Problems of this sort can only be listed at the present time and left to await the results of the research interest this subject merits.

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Seed germination

10

A mature seed contains an embryo and stored food enclosed in one or two seed coats. One seed coat is invariably hard and woody. When a seed has two coats, the inner one is little more than an extremely thin membrane. The embryo is the potential plant and develops from the fertilized egg, while the seed is a part of the parent. The growth of this embryo is arrested in most mature seeds, and the embryo remains in a state of rest as long as the seed is stored in a cool, dry place. The resumption of growth of the embryo after this dormant period and the emergence of the young plant are called germination.

Since the embryo cannot synthesize its own food, it must depend on stored reserve materials for its nourishment. When a seed is placed in the proper environment, the embryo resumes its growth by utilizing the stored food and eventually becomes a more highly developed structure with some of the characteristics of the parent plant. At this stage of its growth the young plant is called a seedling.

CHEMICAL COMPOSITION OF SEEDS

Seeds contain variable quantities of the elements and compounds that are necessary for the formation of the new tissues characteristic of the seedling. Organic compounds such as lipides, proteins, and carbohydrates predominate. Various substances, in addition to those found in the three large classes of compounds just mentioned, are also present. Both macro- and micronutrient elements are present in sufficient amounts to assure the growth of the young seedling to the point where it may secure these elements from soil or the nutrient medium in which it is grown.

The composition of seeds produced by one plant species under similar environmental conditions is quite constant, although seeds of different varieties differ slightly. Marked variations are found in the compositions of seeds of different plant species. For example, soybeans are rich in protein; wheat, oats, corn, and barley contain large amounts of starch; flaxseed and peanuts have a high fat content.

TABLE 10–1. Chemical Composition of Seeds

Kind of Seed	Water, %	Ash, %	Crude Protein, %	Crude Fiber, %	N-free Extract, %	Lipides, %
Barley	10.6	2.8	12.7	5.4	66.6	1.9
Corn, dent No. 1	13.0	1.2	8.8	2.1	70.9	4.0
Cottonseed	9.4	4.6	19.5	22.6	24.9	19.0
Flaxseed	6.2	3.6	24.0	6.3	24.0	35.9
Oats	9.8	4.0	12.0	11.0	58.8	4.6
Peanut kernels	5.4	2.3	30.4	2.5	11.7	47.7
Soybeans	10.0	4.6	37.9	5.0	24.5	18.0
Wheat	10.5	1.9	13.2	2.6	69.9	1.9

EFFECT OF ENVIRONMENT ON SEED GERMINATION

Certain external conditions are necessary for the germination of a seed. Among these are adequate moisture, sufficient oxygen, and a suitable temperature. For the seeds of some species, light is also of importance to the germination process.

Moisture

The water content of stored seeds of the common agricultural plants is usually about 5 to 12 per cent. Since this moisture content is too low to allow rapid metabolism, the first step in the germination of these seeds must be an increase in water content.

Many of the chemical compounds present in embryos, storage organs, and some seed coats have a marked affinity for water. Some seeds have such a strong attraction for water that they can secure a sufficient amount of moisture for germination from an air-dry soil. Most seeds can even pass through the earlier stages of germination if they are placed in an atmosphere which is nearly saturated with water vapor. In general, therefore, the moisture content of the soil does not have to be at soil capacity for germination to occur. However, the affinity of most seeds for water is insufficient to secure adequate moisture for germination from soils of high osmotic concentration. For this reason high concentrations of soluble fertilizers should not be placed close to germinating seeds.

Some seeds, such as red clover, possess hard, impervious coats which do not allow water to pass. These seeds are frequently prepared for germination by treatment with sulfuric acid, or they are blown against needle points which scratch or puncture the outer coat. Treatment with $ZnCl_2$ in HCl (Cross and Bevan's solution), extraction with fat solvents, and many other methods have been used to remove obstacles to the penetration of water.

Seeds respire during storage even though this process takes place at a low rate. One of the factors which determines the rate of this respiration is the moisture content of the resting seed. If seeds are stored under conditions of relatively high humidity, their vitality may be seriously impaired. The critical moisture content of stored seeds depends on the temperature. As the temperature is lowered, the moisture content can be increased without harmful effects.

Temperature

The optimal temperature range for the germination of seeds of most agricultural plants seems to be from 68 to 86°F. Some seeds, such as those of celery, bluegrass, and redtop, germinate better under certain alterations of temperature than at constant temperatures. Most seeds fail to germinate at temperatures as low as 32°F., although a period of moist storage at temperatures of 35 to 55°F. results in better germination of many seeds. Low-temperature treatment of seeds often results in a more rapid and vigorous growth of the seedlings. Although the effect of such low-temperature treatment is well known, the reason why a seed so treated will later germinate more rapidly and grow better is not known. Temperatures as high as 110°F. prevent the germination of most seeds.

Oxygen

Seeds have characteristic requirements as to the amount of oxygen needed for germination. Most seeds germinate in the presence of air, whereas others, such as those of cattails, germinate poorly or not at all unless the oxygen supply is reduced. As a rule, any condition leading to a lack of oxygen and an accumulation of carbon dioxide is harmful to germination. In some cases the germination process is hindered by the presence of a few hundredths of a part of carbon dioxide in one part of oxygen. A thick covering of compact soil may bring about such a condition. The thickness of the soil cover which is best for germination depends upon the physical condition and the moisture content of the soil as well as the kind of seed.

An interesting example of delayed germination due to a lack of oxygen is found in the cocklebur. Each cocklebur contains two seeds; the lower of the two germinates the first year after ripening, whereas the upper seed may germinate a year or more later. A thin membrane surrounding the upper seed prevents sufficient oxygen from reaching the embryo and results in delayed germination. This membrane must be made more permeable to oxygen before germination will take place. Freezing, decay, or high temperatures will accomplish this. Other seeds are also known that lie dormant in the soil because of lack of oxygen, for, although they absorb moisture readily, they possess membranes which reduce the oxygen supply to the embryo. Viable seeds respire during storage, but such respiration is very slow, for there are records of seeds that were still alive after many years of storage. Thus the seeds of *Nelumbium* (a species of large water lily) were alive after storage for 250 years. Since the respiration rate of such long-lived seeds is very low, only minute amounts of oxygen are required during their storage.

Light

The effect of light on germination varies considerably with seeds of different species. For the seeds of the onion and many other members of the lily family the presence of light has an inhibitory effect on the germination process. The seeds of several common agricultural plants, such as corn, small grains, clover, and beans, germinate equally well in the presence or the absence of light. For the seeds of many grasses, the evening primrose, and mullein, light is a desirable adjunct to the germination process. Finally, seeds of the strangling fig, mistletoe, and some other epiphytes fail to germinate unless exposed to light.

PHYSIOLOGICAL FACTORS IN SEED GERMINATION

Age of the Seed

Seeds degenerate with age, although a certain percentage of various seeds will germinate after storage for many years. The reason for this loss of vitality is probably not the depletion of stored foods, for even aged seeds contain large amounts of reserve food material. However, changes in the protein fraction of seeds take place. A decrease in true protein content and an increase in low molecular-weight compounds such as amines, amides, and amino acids have been shown to occur. This has been interpreted as indicating that the longevity of seeds depends on the retention of the original structure of their proteins. As soon as too many of the original proteins have degenerated, it is no longer possible for the seed to germinate since it cannot form the new nitrogen compounds, particularly proteins, essential for the development of the embryo. This theory is unproved but at least has some grounds for credibility. If protein degeneration results in the production of compounds from which the proteins of enzymes can no longer be formed, the seed will not germinate.

Inhibitors

Some freshly harvested seeds contain compounds that check or even inhibit seed germination. During dry storage of the seed, these inhibitors either volatilize or decompose, so that, when the seed is exposed to the proper environment, germination will occur. Inhibitors occur in the coats of fresh lettuce seeds, in the woody materials of the seed balls of beets, and in the fleshy fruits of tomato and cucumber. The fact that seeds will not germinate while in fruit, but retain their ability to germinate after the fruit covering has been removed, is explained by the presence of inhibitors. These chemical inhibitors include such substances as ammonia, hydrocyanic acid, coumarin, parascorbic acid, essential oils, and some alkaloids.

Special Seed Treatments

Treatment of seeds in order to control certain plant diseases, particularly diseases caused by fungi, has been practiced for many years. Copper sulfate was used as a seed fungicide as early as 1761. Since that time many other copper compounds, including the carbonate, oxide, chloride, oxychloride, and arsenate, have been used with varying degrees of success. Copper compounds have been particularly effective in controlling such diseases as bunt and stinking smut of wheat.

Many inorganic and organic compounds of mercury have been used to control diseases of seeds and tubers. Mercuric chloride, for example, has been employed extensively for the treatment of seed potatoes to prevent scab and for the treatment of wheat to control the *Fusarium* organism. Organic mercurials have been used to treat corn, peas, wheat, oats, and other seeds. Many commercial preparations, such as Ceresan, New Improved Ceresan, Semesan, Special Semesan, Upsulum, and Agrox, are available in this country and are valuable as seed fungicides. Other organic fungicides, including formaldehyde, paraformaldehyde, chloranil, dichlone, phenols, and hexachlorobenzene, have also been used.

In general, fungicides have an inhibitory effect on enzyme action, and most of the fungicidal agents exert an unfavorable effect on seed germination. It is generally believed that a substance must enter tissues in order to be toxic to an organism. There is evidence that a number of fungicides penetrate seeds. Thus it has been found that potatoes sprayed with Bordeaux mixture contain more copper within the tuber than untreated potatoes. It has been noted that seeds with damaged seed coats are more likely to be killed or inhibited after treatment than the same variety with intact seed coats.

In most cases fungicidal treatment of seeds seems to increase the time needed for complete germination. The yields of agricultural crops from treated seed are often depressed unless the seeding rate is increased. It has been reported that certain seed treatments stimulate the germination process, but usually the benefits derived from seed treatment are due to control of organisms found either on the seed or in the seed bed and not to stimulation of germination.

METABOLISM OF GERMINATING SEEDS

When seeds are placed in an environment favorable to germination, the rate of metabolism is markedly accelerated. The reactions taking place include hydrolysis, oxidation, desmolysis, and synthesis. Stored food is changed from insoluble, immovable substances to soluble, transportable compounds which are translocated. With these soluble compounds as building blocks, the embryo can synthesize compounds needed for the manufacture of new tissue. There is a great increase in enzyme activity of seeds during germination. Carbohydrases, proteinases, lipases, oxidases, and desmolases are active in germinating seeds.

Carbohydrate Metabolism

The main storage carbohydrate of seeds is starch. The amylose and amylopectin components of starch are broken down by amylases or by phosphorylase. The result of the hydrolysis of starch by amylases is a mixture of glucose, maltose, and small dextrins. Maltase hydrolyzes the maltose to glucose. Low-molecular-weight dextrins are also hydrolyzed to glucose, but the method by which this is accomplished is uncertain. Glucose produced by amylase and maltase action is converted to glucose-6-phosphate by hexokinase. A more detailed discussion of this and succeeding processes appears in Chapter 7.

Peas, beans, and potatoes are rich in phosphorylase. Most seeds probably contain this starch-splitting and starch-forming enzyme. Phosphorylase converts starch to glucose-1-phosphate. Glucose-1-phosphate is changed to glucose-6-phosphate by phosphoglucomutase. Thus starch hydrolysis, by means of amylases, maltase, and hexokinase or by phosphorylase and phosphoglucomutase, results in the formation of the same compound, glucose-6-phosphate.

Glucose-6-phosphate is transformed by a series of changes which is discussed on pages 168 to 173. This process involves splitting a hexosephosphate to two triosephosphates and oxidation of the latter to pyruvic acid. Pyruvic acid may be oxidized to carbon dioxide and water through the Krebs cycle. The intermediary compounds and the Krebs cycle acids can be used for the synthesis of fats, proteins, or new carbohydrates required for the growth of the embryo and seedling.

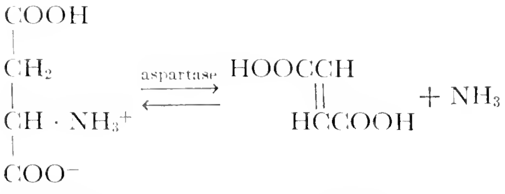
Some germinating seeds, for example, barley, contain large amounts of sucrose. It is now known that sucrose can be formed by the reactions found on page 220.

In germinating seeds, it is probable that fructose or a fructose phosphate is necessary for the formation of sucrose. Fructose phosphates are produced in the breakdown of starch. Fructose is produced by the hydrolysis of fructose-6-phosphate by a phosphatase. Sucrose in seeds can be hydrolyzed to glucose and fructose by the action of sucrase. This reaction was discussed in Chapter 6 on enzymes.

Protein Metabolism

Protein reserves of the seed are rapidly changed into simpler compounds during germination, and these are translocated to the point of utilization. Protein breakdown is initiated by proteinases of seeds. These plant enzymes belong to the papainase group, which is inactive when in the oxidized form but is activated by reducing agents. Papainases hydrolyze proteins to mixtures of polypeptides and small amounts of amino acids. The polypeptides are then hydrolyzed to amino acids through the action of peptidases.

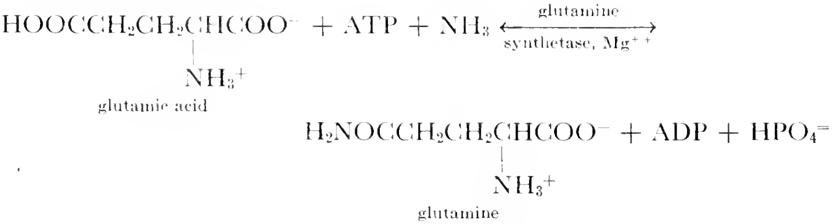
The amino acids may be utilized for the production of essential plant compounds such as enzymes and nucleoproteins or for the formation of other compounds such as porphyrins. Amino acids can be deaminized by deaminases (aminases), amino acid oxidases, or transaminases. An illustration of the deamination of an amino acid by deaminases of seeds is the action of aspartase.



$$\begin{array}{l} COOH \\ | \\ CH_2 \\ | \\ CH\cdot NH_3^+ \\ | \\ COO^- \end{array} \overset{\text{aspartase}}{\rightleftarrows} \begin{array}{c} HOOCCH \\ \| \\ HCCOOH \end{array} + NH_3$$

aspartic acid fumaric acid

The ammonia produced can react with organic acids to form amides. When ammonia reacts with a molecule of glutamic acid in the presence of ATP, Mg^{++}, and glutamine synthetase, glutamine is formed.



$$\underset{\text{glutamic acid}}{HOOCCH_2CH_2\underset{\underset{NH_3^+}{|}}{C}HCOO^-} + ATP + NH_3 \underset{\text{synthetase, Mg}^{++}}{\overset{\text{glutamine}}{\longleftrightarrow}} \underset{\text{glutamine}}{H_2NOCCH_2CH_2\underset{\underset{NH_3^+}{|}}{C}HCOO^-} + ADP + HPO_4^=$$

Asparagine and glutamine are excellent carriers of nitrogen for protein synthesis. These compounds can be broken down to give products available for the synthesis of the particular amino acids and proteins required for growth of an embryo.

Amino acid oxidases produce ammonia, hydrogen peroxide, and keto acids from amino acids. Keto acids also result when amino acids lose their amino groups by transamination. The keto acids can be decarboxylated, forming the corresponding aldehydes, or they may be utilized through the pathway of the Krebs cycle transformations.

Fat Metabolism

The fats and oils of mature seeds are found in relatively large globules. During the resting period and during germination these globules are reduced in size, thus providing more surface for the action of fat-splitting enzymes.

In the presence of sufficient water, the lipases of seeds rapidly hydrolyze the fats and oils to glycerol and fatty acids. Neither the fatty acids nor the glycerol accumulate in large concentrations during germination but are quickly changed to other compounds. The principal products formed from fats during the germination have been shown to be carbohydrates.

A suggested mechanism for the conversion of fats into carbohydrates is as follows: (1) Fats and oils are hydrolyzed by lipases to fatty acids and glycerol. (2) Fatty acids are oxidized by fatty acid oxidases to acyl CoA compounds. (3) Acetyl CoA reacts with oxaloacetic acid to form citric acid. (4) Citric acid is changed by way of the Krebs cycle to carbohydrates by the reactions of carbohydrate metabolism, as shown in Chapter 9. (5) Glycerol is converted to α-glycerophosphate. (6) The α-glycerophosphate is dehydrogenated to triosephosphate. (7) Triosephosphates combine to form hexosephosphates.

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Plant nutrition

11

ESSENTIAL NUTRIENTS

Water

There is no doubt that water serves as one of the most important plant nutrients. Water is the most abundant ingredient of active plant cells, and comprises 85 to 95 per cent of the fresh weight of actively growing tissue. On the other hand, the water content of dormant structures, such as seeds, may be as low as 5 to 10 per cent. As has been discussed in Chapter 2, water has a number of properties which make it an important physiological substance. In so far as the plant is concerned, water is (1) an important raw material for the synthesis of organic compounds, (2) the medium in which solutes are dispersed and through which such solutes move from cell to cell, and (3) the solvent or dispersion medium in which vital chemical reactions take place. Moreover, water gives turgor to plant cells.

Water moves upward in the plant, from the roots to the stems and then to the leaves, where a portion of it is lost to the atmosphere as water vapor. The final process is known as transpiration and accounts for a large percentage of the water taken up by a plant during its lifetime. The amount of water transpired by a plant during its lifetime is very large, varying between 200 and 1000 times its dry weight. Such loss cannot be construed as wasteful, however, since transpiration is the end to a means of circulation in a plant. Hence many of the soluble nutrients are translocated within the plant during this process of water movement.

A number of factors influence the rate of transpiration in plants, among which may be mentioned atmospheric humidity, air temperature, air movements, light intensity, and soil conditions. Such morphological factors as distribution and placement of stomata, thickness and character of cuticle, and amount of leaf surface play important parts in the rate of transpiration.

Carbon Dioxide

The role of carbon dioxide in photosynthesis has been discussed in Chapter 9. It has been established that the growth of a plant is related to the amount of carbon dioxide available to it for photosynthetic purposes. Experiments have shown that most plants manufacture more carbohydrates and grow faster if exposed to higher-than-normal amounts of carbon dioxide. However, over an extended period of time an elevated percentage of carbon dioxide in the atmosphere will be deleterious to the plant. Carbon dioxide is given off by plant roots and is known to play an important part in plant-soil relationships.

Oxygen

The requirement for oxygen during biological oxidation and the production of oxygen in the photosynthetic process have been discussed in Chapters 7 and 9, respectively. Thus it can be seen that, while the plant releases oxygen as a by-product of the photosynthetic reaction, the same plant has oxygen requirements for the biological oxidations taking place within it. This need for oxygen is greatest at night when the rate of photosynthesis is nil, and the majority of reactions taking place are enzyme-catalyzed energy-releasing reactions. Such reactions require molecular oxygen for the final transfer of electrons and the formation of water.

MINERAL ELEMENTS

Although a large number of elements have been shown to exist in plant tissues, only fifteen of these have been proved to be essential for higher plants. The roles of carbon, hydrogen, oxygen, and nitrogen in the synthesis of carbohydrates, lipides, proteins, and related compounds have been discussed in preceding chapters. The essential elements that remain to be discussed are usually classified as the mineral elements and include phosphorus, sulfur, calcium, magnesium, potassium, iron, boron, manganese, copper, zinc, and molybdenum.

The precise functions of many of the mineral elements in plant metabolism are not completely understood. Whereas often it is relatively easy to identify the conspicuous role played by some elements, it is difficult to state that the same element does not have a number of minor functions in plant metabolism. By the same token, it is reasonable to assume that an element might play a series of important but inconspicuous roles and therefore not be recognized as an essential element. The elements included in the following discussion are known to have specific functions in plant metabolism.

Phosphorus

This element, which is absorbed by plants chiefly as the $HPO_4^{=}$ ion, is not reduced in plant tissues but rather is incorporated into organic compounds in highly oxidized form. Phosphorus, as the phosphate, is combined as part of the phospholipide and the nucleic acid molecules. The significance of phosphate carriers such as ATP and ADP and the importance of phosphorylation reactions in living tissue have already been discussed.

In the growing plant, a large proportion of the phosphorus is found associated with meristematic tissue, where it is used in the synthesis of nucleoproteins and some other phosphorus-containing compounds used in the respiration mechanism. In growing plants phosphorus is easily redistributed from one organ to another. Such redistribution is most evident under conditions of phosphorus deficiency of the medium in which the plant is grown. At such times, phosphorus, probably as the phosphate, is drained from mature leaves into other tissues. Under such conditions of phosphorus deficiency in the medium, the phosphate of even the youngest leaves is sacrificed in the formation of the seed or fruit of the plant.

In mature plants, a large proportion of the phosphorus accumulates in the seed or fruit as a result of their development. In fact it has been shown that phosphorus favors seed formation and maturation of plants. In this respect it is antagonistic to nitrogen, which favors vegetative growth and delayed maturity. Because of its limited solubility in the soil solution, an excessive phosphorus content is seldom a problem. However, low phosphorus not only affects plant growth and metabolism but also gives rise to phosphorus deficiency in animals who subsist on such plants.

Sulfur

This element is found widely distributed among the plant tissues. It is an essential component of two important amino acids, cystine and methionine. It is also a structural component of biotin and thiamine, two important plant hormones. Sulfur also forms a part of the mustard oil glycosides, which are responsible for imparting characteristic odor and flavor to the mustards, onions, and garlic.

Although sulfur can enter the leaves as SO_2 when this gas is in the atmosphere, the usual route of entry for the element is through the roots as the sulfate ion. Before combination in the organic compounds mentioned above, the sulfate ion is reduced, usually to the sulfhydryl (—SH) group. Once combined in organic form, the sulfur apparently can be reconverted to inorganic sulfur, usually the sulfate ion, and thus be redistributed within the plant and used again as a source of sulfur for the synthesis of other organic compounds.

Sulfur is also known to play an important role in the physiology of plants. A deficiency of sulfur interferes with the production of chlorophyll, resulting in the leaves appearing pale green, as in nitrogen deficiency.

Calcium

Much of the calcium in most plants is located in the leaves. In contrast to phosphorus, more calcium is generally found in older rather than in younger leaves. It is believed that much of the calcium in plants is permanently fixed in cell walls as the calcium salt of the pectic substances, or as calcium oxalate. Calcium is also widely distributed in the plant in the form of calcium soaps and is present in vacuolar sap as calcium ions.

Calcium has been found necessary for continued mitotic division. It is also known to play an important role in the nitrogen metabolism of some plants, for a deficiency of calcium leads to a poor absorption or assimilation of nitrates in such plants.

Unlike phosphorus, calcium is not readily redistributed in a plant when the soil is deficient in this element. Although redistribution apparently takes place at a very slow rate, such translocation is not sufficient to meet the metabolic needs of the younger, rapidly growing tissues.

Magnesium

Although seeds contain an appreciable amount of magnesium, the largest proportion of this element present in plants is found in the chlorophyll-bearing tissues. Most soils contain a sufficient amount of magnesium to supply the requirements of plants. In the event that an adequate supply is not available to the plant, the production of chlorophyll is inhibited and a condition known as chlorosis obtained. Under deficiency conditions, magnesium is readily redistributed from the older to the younger plant tissues.

Magnesium plays a part in the phosphate metabolism of plants and therefore assists in the respiratory mechanism. Magnesium ions apparently are specific activators for a number of enzymes, such as some carboxylases and transphosphorylases.

Potassium

Potassium has a number of important functions in plant metabolism, although this element is not definitely known to be a constituent of any organic compounds essential to plant life. It is nevertheless indispensable and is generally present in soluble inorganic salts as well as in organic salts. Owing to the fact that potassium is highly mobile, it is invariably found in all sap and plays an important part in the water economy of plants through the effect of its soluble compounds on osmotic pressure.

Potassium aids the oxidative reactions of plants by acting as a carrier for iron, which in turn is required by a number of important oxidative enzymes. The assimilation of carbon and the formation of carbohydrates have been shown to be dependent on the presence of potassium. One of its functions in carbohydrate metabolism is to form the potassium salts of the phosphorylated intermediates. High-carbohydrate plants such as sugar beets have a high potassium content. Protein metabolism in plants also requires the presence of an ample supply of potassium.

In the early stages of growth, plants supplied with limited amounts of potassium may show normal development. Later the relatively small amount of potassium migrates from older tissues to younger tissues. This is followed by a migration of nitrogen to the younger tissues. Such migrations result in premature aging of older leaves when potassium is present in suboptimal amounts.

Iron

A deficiency of iron in soils is usually a result of its insolubility and hence its unavailability, rather than its actual absence. It is far more available in acid soils than in neutral or alkaline soils. Even in the latter case, however, some iron absorption takes place as a result of intimate contact between roots and soil particles. Thus a deficiency of iron is seldom a limiting factor in plant production.

Iron is indispensable to the synthesis of chlorophyll. Thus a deficiency of iron in plants results in a lack of chlorophyll. In spite of its importance in the formation of chlorophyll, iron does not become a part of this complex molecule. It seems clear, however, that in its stimulatory role iron is most effective as the Fe++ ion. Although this element is frequently absorbed in the ferric state, it is rather rapidly reduced within the cells to the ferrous condition, in which form it is said to be physiologically active.

Iron is known to be intimately associated with a number of the enzymes and carriers which function in the oxidative mechanism of living cells. For example, iron is an important ingredient of catalase, peroxidase, and the cytochromes.

Despite its importance, the total amount of iron found in plant tissues is very low and much of that which is present is in organic combination. Unlike a number of the other elements, iron is quite immobile in the plant; hence very little redistribution of iron reserves takes place. This can be demonstrated quite readily by removing a plant from an environment in which it has been supplied with iron and placing it in a cultural solution deficient in this element. Whereas the old leaves retain their normal green color, new leaves will show distinct symptoms of chlorosis. Hence no appreciable transfer of iron takes place from older to younger tissue.

Boron

The exact role that boron plays in plant metabolism is unknown. The accumulation of carbohydrates, ammonium compounds, and other soluble nitrogenous substances in boron-deficient plants suggests a block of the protein-synthesis mechanism. Leaf-roll of potato, browning of cauliflower, and brown-heart of turnip are diseases of these plants which have been identified with boron deficiency.

While boron is necessary only in very small quantities, there seems to be quite a variation in the requirements of some species when compared with others. For example, whereas tomatoes seem to develop best on boron concentrations of less than 1 p.p.m., asparagus exhibits its greatest development when boron is available to the extent of 10 to 15 p.p.m. Curiously enough, the range between the amount of boron necessary for optimal plant development and the amount causing injury is very small and the quantities sometimes overlap. Thus, when some plants are supplied with sufficient boron for optimal development, they may exhibit boron-toxicity symptoms at the same time.

Manganese

Except in extremely low concentrations, manganese is very toxic to plants. Like iron, manganese is not often deficient in soils but becomes unavailable to plants under alkaline conditions. Once inside a plant, this element is generally located in the more physiologically active parts, particularly the leaves. Since it is a relatively immobile element, manganese is not readily redistributed throughout the plant.

Manganese undoubtedly plays a number of roles in the plant. It has been shown that Mn^{++} is an activator of some enzyme systems, including some carboxylases and dehydrogenases. This element is also related in some way to chlorophyll synthesis, since a deficiency of Mn^{++} leads to a chlorosis distinctly different from that obtaining as a result of iron or magnesium deficiency. Manganese also probably plays a part in oxidation-reduction reactions in the plant. It is known, for example, that excess manganese acts as an oxidizing agent, converting ferrous ion to Fe^{+++}, thereby effectively inducing symptoms of iron deficiency by converting Fe^{++} to the physiologically inactive ferric state.

Copper

This element is highly toxic to plants except in very low concentration. However, there is no doubt that traces of copper are essential for plant development. This element acts as part of certain oxidizing-reducing enzymes such as tyrosinase and ascorbic acid oxidase. Aside from this particular function, no specific role for copper has been established in the plant.

Zinc

Zinc is known to be a part of the enzyme, carbonic anhydrase. Although highly toxic even in moderate concentrations, traces of this element are required for normal plant growth. For instance, it is fairly well established that zinc is necessary for the synthesis of one of the plant-growth hormones, indoleacetic acid. Zinc also functions in the active DPN^{+} complex during reactions catalyzed by alcohol dehydrogenase.

Molybdenum

Of all the essential elements, molybdenum is required in the smallest concentration for plant growth and development. For example, it has been demonstrated that 1 part in 100,000,000 parts of culture solution is sufficient to prevent molybdenum deficiency in tomato plants. Although the complete role that molybdenum plays in plant development is not understood, it is generally agreed that this element functions in the reduction of nitrates.

ROLE OF THE SOIL

Soil Formation

Soil is the loose, easily crumbled part of the earth's crust in which plants find support and nourishment. This loose, friable material is a mixture of four major components, inorganic material, organic matter, water, and air. The process by which this mixture is formed and becomes a suitable medium for plant nutrition is complex. A detailed consideration of soil formation is beyond the scope of this discussion. However, the principal forces involved in the formation of soil include physical forces causing disintegration and mechanical subdivision of rocks and rock particles, chemical forces causing decomposition, and biological forces resulting in both disintegration and decomposition.

The physical forces acting on parent rock and smaller particles include the following: changes of temperature, causing expansion and contraction; freezing of water in cracks and crevices; abrasion when boulders, rocks, gravel, and sand are carried by streams of water; wind-borne sand, which grinds away native rock and moves particles to new locations; and glaciers, which pick up rock and rock particles and cut, grind, and crush native rock over which they move.

Simple solution is probably the first reaction which takes place between water and any kind of rock mineral. Among the materials most readily dissolved are compounds of sodium, potassium, calcium, and magnesium. When water dissolves a mineral such as calcium silicate, hydrolysis takes place with the formation of calcium hydroxide and silicic acid.

$$CaSiO_3 + 2H_2O \rightleftharpoons Ca(OH)_2 + H_2SiO_3$$

The silicic acid may lose water, thereby forming SiO_2, a very unreactive compound. The calcium hydroxide may be washed away as such, but it is more likely to be changed by carbonation to calcium carbonate. Carbonic acid, the carbonating agent, may arise from the carbon dioxide produced by plant roots, by decomposing organic matter or by microorganisms, from carbon dioxide in the air, or from carbonates of rocks and minerals. Carbonation affects compounds containing sodium, calcium, potassium, and ferrous iron.

Hydration plays an important part in chemical decomposition by forming hydrated products of minerals. A large number of silicates, oxides, and carbonates will form hydrated compounds. These compounds are more reactive than the original complexes.

Most of the minerals found in rocks are already fully oxidized. Exceptions include minerals containing ferrous iron, for example pyrites, which can be changed to ferric compounds by oxidation. This process requires oxygen and water and takes place more rapidly in humid than in dry regions. Oxidation of elements such as sulfur and nitrogen leads to the formation of mineral acids which have a solvent action on rock constituents.

$$2FeS_2 + 7H_2O + 15\ O \rightarrow 2Fe(OH)_3 + 4H_2SO_4$$

Rocks may be broken by the expansion of plant roots in cracks. Mosses and lichens produce disintegration when they grow in intimate contact with rock and mineral surfaces. Various microorganisms are active in the decomposition of parent soil constituents. Animals such as rodents and earthworms aid in mixing various horizons of soil and in supplying fresh subsoil to surface layers.

When sufficient soil has been created to supply a substrate for higher plants, soil formation is accelerated. The roots of these plants contribute carbon dioxide and, when they die, organic matter which in turn acts as substrate for microorganisms. Products of microorganism metabolism, including carbon dioxide, ammonia, and organic acids, hasten the decomposition of rock material. The intimate relationship between the resulting soil and the animal world is an essential feature of life on this planet.

Inorganic Matter in Soils

Most of the chemical elements known to man may be expected to exist in the soil, and a large number of them have been found to be present. However, the greater part of the inorganic matter in soil is composed of relatively few elements.

The approximate average composition of soils suitable for agriculture is as follows:

Element	Oxide	Percentage
Silicon	SiO_2	78
Aluminum	Al_2O_3	10
Iron	Fe_2O_3	5
Potassium	K_2O	2
Calcium	CaO	1
Magnesium	MgO	1
Sodium	Na_2O	1
Titanium	TiO_2	1
Others		1
		100

The composition of crop-producing soils may vary widely from the average given above. Thus American agricultural soils have been found to contain as low as 11 per cent and as high as 97 per cent silica. Aluminum oxide has been found to vary from below 2 per cent to 27 per cent. All soils contain small quantities of many elements not listed above, including manganese, phosphorus, and sulfur.

The composition of soils is usually expressed in terms of the oxides of the elements. However, the assumption should not be made that all elements exist as their oxides in soil. The only common oxide present in large amounts is the oxide of silicon, SiO_2. Most elements are present as parts of complex compounds such as iron or aluminum silicates.

A study of soils and their composition has shown that there are four main groups of constituents important in determining the nature of a soil. The predominance of one or more of these four constituents will establish certain characteristics of a soil. Organic matter is one important constituent; the other three are groups of inorganic compounds:

1. Compounds of silicon, usually called silica.
2. Compounds of alkali and alkaline-earth metals. The important elements are Na, K, Mg, and Ca.
3. Compounds of iron and aluminum, referred to as the sesquioxides of iron and aluminum, for example, Fe_2O_3 and Al_2O_3.

Soils high in silica but low in the other two groups of inorganic constituents are usually acid. Such acid soils occur in cool, humid climates and are generally open-textured, sandy, gravelly, or stony. These cold-climate soils may contain large amounts of organic matter, often only semidecomposed. The soils of cold and moist climates, called "gray earths," "forest soils," or "podzols," belong in the high-silica group.

Arid climates, whether hot or cold, allow the accumulation of alkali and alkaline-earth compounds in soils. If evaporation of water equals or exceeds rainfall, there is little percolation of water through the soils and very little leaching of soluble compounds. Under these conditions alkali and alkaline-earth metals accumulate as carbonates and other basic compounds. As a rule such alkaline soils are low in organic matter. Irrigation of many arid-climate soils results in the production of crops with very large yields.

Under cool, semiarid conditions organic matter and calcium accumulate, but the alkali metals, sodium and potassium, are not present in large amounts as basic compounds. Such soils, found in the wheat-growing areas of western United States and Canada, are among the most fertile soils in the world. The black prairie grass soils belong to this group.

A balanced distribution of organic matter, sesquioxides, alkaline-earth compounds, and silica is found in humid climate soils of central Europe and central-eastern United States. Soils of these regions have a tendency to be slightly acid.

South temperate, subtropical, and tropical soils in humid climates are high in iron and aluminum. They contain less organic matter and less silica than soils in cooler climates, and they are usually low in calcium and magnesium. In such soils, called laterites, compounds of iron and aluminum are dominant. In moist tropical climates organic matter decomposes rapidly in the soil, producing CO_2. The carbonates which are formed attack silica, forming more soluble compounds which are removed by leaching. Since alkali and alkaline-earth compounds also are rapidly lost, the predominant compounds become the iron and aluminum group.

Organic Matter in Soils

Soil organic matter is composed of the bodies of dead organisms and the excretions of living organisms deposited on or in the soil. Theoretically soil organic matter consists of only the non-living remains of organisms and the products of their decomposition. It is impossible to separate such material from the living microorganisms that inhabit the soil and are the most important agents in the decomposition of dead material. The bodies of living microorganisms are therefore commonly included as part of the soil organic matter. Thus our soil organic matter consists of dead roots, leaves, fruits, and stems of plants; carcasses of worms, insects, and animals; bacteria, fungi, and protozoa; and decomposition products of dead organisms.

Since the soil organic matter is a mixture of plant and animal material, the chemical compounds present include carbohydrates, proteins, lipides, and other compounds found in plants and animals. The gradual decomposition of these compounds ultimately leads to the formation of such products as water, carbon dioxide, ammonia, methane, and simple inorganic salts. But before these simple end products are formed, many intermediate compounds have been produced, some of them of less complexity than the original compounds, others of greater complexity.

The mixture of compounds resistant to decay formed during the decomposition of organic compounds in the soil is called humus. Soil organic matter is called humus when decomposition has proceeded to the stage where the specific structure of the original organized tissue has disappeared. Humus is characterized as an amorphous, dark-colored, nearly odorless material, having no definite chemical composition.

The carbon content of humus, about 55 to 58 per cent, is greater than the carbon content of the original plant and animal matter from which it is formed. The nitrogen content of humus is variable, usually falling in the range of 3 to 6 per cent. The ratio of carbon to nitrogen is therefore between 20 to 1 and 10 to 1, depending on the kind of organic matter from which it was derived, the stage of decomposition, the nature and depth of the soil, and the climatic conditions under which it was formed.

Since humus originates from the decomposition of plant, animal, and microbial residues, compounds are found in it which are similar to, or derived from, the compounds of the parent material. Thus significant amounts of the following four groups of compounds are found: (1) Lipides and related materials. Fats, waxes, higher alcohols, resins, sterols, organic acids, and pigments are found in rather small amounts. (2) Carbohydrates and their derivatives. Polysaccharides such as cellulose, pentosans, hexosans, polyuronides, glycosides, starch, and sugars are present in varying amounts. (3) Proteins and other nitrogen compounds. This group of compounds, which includes proteins, proteoses, peptones, amino acids, amines, purines, and pyrimidine bases, is present in fairly large amounts. (4) Lignin (see page 186) and its derivatives. Lignin is one of the most stable compounds found in plants. It constitutes 25 to 30 per cent of the wood of trees and 15 to 20 per cent of straw. Lignin is present in large amounts in humus. Thus the two major groups of compounds found in humus are proteins and lignins. Probably most of the humus found in soils results from a combination of lignin with bacterial proteins and is commonly known as a lignoproteinate.

Humus is nearly insoluble in water, but a part of it is soluble in dilute alkali solutions. Many of the important properties of humus depend on the fact that, alone or with inorganic substances of the soil, it forms complex colloidal systems. The properties and importance of soil colloids will be discussed later.

Two important groups of humus substances have been recognized: (1) nutrient or temporary humus, which is easily broken down and which serves as a source of carbon for microorganisms; (2) persistent or maintenance humus, which is slowly converted to other substances and forms the important organic colloidal material of soil.

Organic matter has several important functions in soils. It serves as a substrate for the growth of microorganisms and improves the physical condition of the soil by improving texture, moisture-holding capacity, and aeration. Organic matter increases the buffering capacity of soil and combines with inorganic soil constituents to prevent their loss by leaching but releases these substances for the use of plants. Finally, organic matter is the sole storehouse of nitrogen.

The importance of humus for the maintenance of soil productivity has been recognized for many years. Repeated observations have shown that virgin soils containing large amounts of organic matter in the form of humus may yield large crops for the first few years of cultivation. These crop yields gradually decrease with the number of years of cultivation and crop removal. One of the chief reasons for this loss of fertility is the loss of humus from the soil under continuous cropping.

However, the reader should remember that neither humus nor organic matter of any kind is essential to plant growth. Plants can be grown in sand cultures with properly balanced nutrient solutions containing no organic compounds, and plants can be grown in water solutions containing only inorganic compounds. The principal importance of soil organic matter, or humus, is its effect on the soil as a medium for plant growth.

Nitrogen Fixation

Nitrogen is one of the elements essential to the maintenance of soil fertility. The lack of sufficient quantities of available nitrogen in soils has long been recognized as a limiting factor in crop production. An ample supply of readily available nitrogen stimulates vegetative growth of plants.

Most of the nitrogen found in soils occurs in combination with the soil organic matter. While in this form it is comparatively insoluble and not available for plant growth. However, the nitrogen of organic matter may eventually be liberated for utilization by plants through the action of microorganisms. All soils contain a small proportion of soil nitrogen in the form of relatively simple compounds such as amino acids, ammonium salts, and nitrates. It is from these compounds, particularly from ammonium salts and nitrates, that higher plants obtain their nitrogen. The total quantity of nitrogen found in mineral soils is usually quite low, varying from 0.1 to 0.5 per cent.

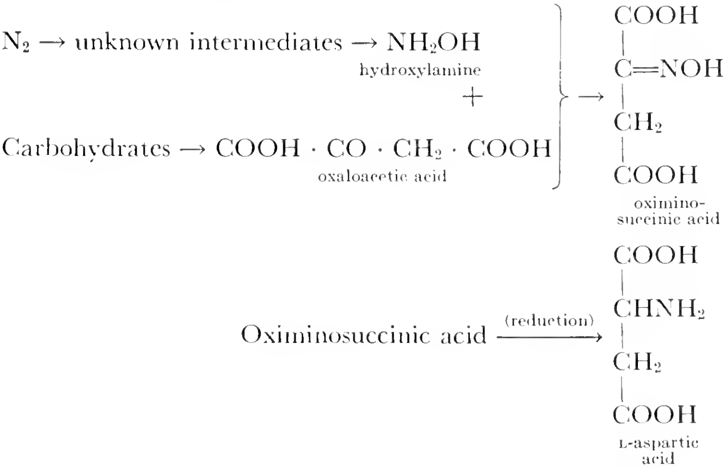
Atmospheric nitrogen is the ultimate source of soil nitrogen. The various processes by which this inert gas is changed into organic and inorganic compounds are biochemical. Some of these processes will now be considered.

Symbiotic nitrogen fixation. It has been known for a long period of time that legumes possess the power of enriching the soil with nitrogen, and that this nitrogen is obtained from the atmosphere through the agency of bacteria living symbiotically upon the legumes. Symbiosis, as applied to nitrogen fixation, is derived from the Greek word for "living together." The bacteria, which belong to the genus *Rhizobium*, live in the root cells of the legume host, where their metabolic activity causes a swelling or nodule to form on the root. For this reason the organisms are commonly called root-nodule bacteria.

One of the facts established concerning the manner in which nitrogen is fixed is that the nodules excrete nitrogen chiefly as aspartic acid, glutamic acid, and β-alanine. Aspartic acid alone constitutes over half of the total in young cultures. As the culture ages the percentage of aspartic acid liberated decreases, while the amount of β-alanine increases. From this fact one can infer that aspartic acid is a primary fixation product, whereas β-alanine is formed by the decarboxylation of the aspartic acid.

It has also been suggested that hydroxylamine is one of the products formed, and that carbohydrate metabolism is closely related to nitrogen fixation. On the basis of these three facts a theory has been advanced which postulates that hydroxylamine units with oxaloacetic acid to form an oxime which is then reduced to aspartic acid.

According to this theory the mechanism of biological nitrogen fixation would be as follows:



$$\left.\begin{array}{r} N_2 \rightarrow \text{unknown intermediates} \rightarrow \underset{\text{hydroxylamine}}{NH_2OH} \\ + \\ \text{Carbohydrates} \rightarrow \underset{\text{oxaloacetic acid}}{COOH \cdot CO \cdot CH_2 \cdot COOH} \end{array}\right\} \rightarrow \underset{\text{oximino-succinic acid}}{\begin{array}{l} COOH \\ | \\ C{=}NOH \\ | \\ CH_2 \\ | \\ COOH \end{array}}$$

$$\text{Oximinosuccinic acid} \xrightarrow{\text{(reduction)}} \underset{\text{L-aspartic acid}}{\begin{array}{l} COOH \\ | \\ CHNH_2 \\ | \\ CH_2 \\ | \\ COOH \end{array}}$$

Since symbiotic nitrogen-fixing organisms are aerobic in character, a lack of oxygen inhibits nodule formation. The fact that nodules are formed in larger numbers near the surface of the soil is attributed to a greater oxygen supply at higher than at lower depths. Moisture conditions should be optimal for aeration and carbohydrate metabolism of the host plant. The optimal temperature for nitrogen fixation by the symbiotic organisms approximates 20°C., providing that the pH of the environment is close to the neutral point. However, nodules are formed under any soil condition in which the legumes can grow.

Non-symbiotic nitrogen fixation. In addition to the symbiotic nitrogen-fixing organisms, there are two other important groups of nitrogen-fixing soil organisms. These function independently of higher plants. One of these has been given the generic term of *Azotobacter*, the best known species of which is *Azotobacter chroococus.* These organisms are aerobic and will fail to develop under slightly acid conditions.

Another group of non-symbiotic nitrogen-fixing bacteria is known by the species name of *Clostridium pastorianum.* These are anaerobic organisms and appear to function effectively in acid as well as in neutral soils and under conditions that are unfavorable to most species of *Azotobacter.*

Ammonification

The production of ammonia from organic nitrogenous materials of the soils is called ammonification. This process is an intermediate step in the process of nitrate formation by soil microorganisms. The organisms concerned with ammonification are the same fungi, bacteria, and actinomycetes that decompose organic matter in soils. The most important ammonifying organisms are aerobic and require a good supply of oxygen and moisture. The most favorable pH range for their action seems to be 6.0 to 8.0.

Nitrification

The conversion of ammonia nitrogen to nitrate nitrogen in soils is accomplished in two steps by two classes of bacteria. The first step, which is carried out by *Nitrosomonas* and *Nitrosococcus* organisms, changes ammonia to nitrites. The second step is carried out by *Nitrobacter* and oxidizes nitrites to nitrates. The final oxidation to nitrates takes place more rapidly than the formation of ammonia or nitrites, with the result that very little ammonia or nitrite nitrogen is present at any one time. The presence of ammonia nitrogen in amounts more than a few parts per million of soil is evidence that conditions are unfavorable for nitrification. This is commonly the case in acid soils rich in organic matter or in soils where the moisture content is high.

Nitrification is an aerobic process, the work of autotrophic bacteria whose energy is obtained from the oxidation in this change. The minimal, optimal, and maximal temperatures for the process are about 5°C., 25 to 35°C., and 55°C., respectively. Although nitrification proceeds most rapidly in a neutral or a slightly acid medium, it is now known that it can occur, though more slowly, in soils that are definitely on the acid side of neutrality. The presence of moisture is, of course, essential for cell microbial actions.

Soil Colloids

Soil particles are classified arbitrarily according to size. An easily remembered classification is that made by the International Society of Soil Science.

Separate	Diameter Limits, mm.
Coarse sand	2.00–0.20
Fine sand	0.20–0.02
Silt	0.02–0.002
Clay	Below 0.002

The most important of the four groups from the standpoint of soil chemistry as related to plant growth is the clay fraction. It is this fraction which contains the soil colloids.

One of the most interesting properties of a fertile soil is its ability to hold plant nutrients with a force strong enough to prevent rapid loss of these nutrients by leaching, yet weak enough to enable plants to remove them. This property is largely dependent on the presence and properties of soil colloids. Colloidal particles also have an important influence on the physical structure, the water-holding capacity, and the buffer action of soils. Since these factors play an important part in determining the fertility of a soil, the properties of the colloidal fraction must be related to soil fertility. Soil colloids are composed of both organic and inorganic materials.

Organic colloids. Colloidal humus is negatively charged and highly hydrated. It usually exists in the soil in the form of a gel which is mixed with, and coats, inorganic particles. The combined effects of its charge and hydration make it quite stable. As in the case of inorganic colloids, it will adsorb positive ions which can function in base exchange.

Inorganic colloids. The inorganic fraction of soil colloids is composed principally of oxides of silicon, aluminum, and iron, with variable amounts of other basic elements. Since colloidal particles are usually considered to be less than 200 mμ (millimicrons) in diameter, such particles will be found only in the clay fraction of soils. Particles with diameters less than 0.002 mm. (2,000 mμ) are classified as clay. Inorganic soil colloids are thus referred to as clay colloids, although not all the clay fraction is of colloidal size.

We have spoken of the diameter of clay colloids, but this does not mean that the colloids are spherical. Actually they are composed of laminated plates; that is, these particles are built up of several thin sheets of crystalline minerals. Such particles are present in a wide variety of shapes and sizes. Let us assume that a clay particle composed of at least two lamellas can be represented by Figure 11–1. Because of its fine size and laminated structure this clay particle contains a large amount of external and internal surface. This surface is electrically charged and shows a definite electrical potential. The potential (zeta potential) is due to the presence of a double layer of ions called the Helmholtz double layer. The inner layer of ions is a fixed, negatively charged coat which forms an integral part of both the internal and external surfaces of the clay particle. It is the charge of this inner layer of anions which determines the characteristic charge of the colloidal micelle. Since this fixed inner layer has a negative charge in fertile soil, these soil colloids are negative and will migrate to the positive pole under the influence of an electrical current.

The outer layer of ions consists of positive ions which are readily exchanged for other cations. Calcium ions and hydrogen ions are present in greatest number, but potassium, magnesium, sodium, and other ions are also present. All these cations are hydrated, the number of molecules of water associated with each being a characteristic of the

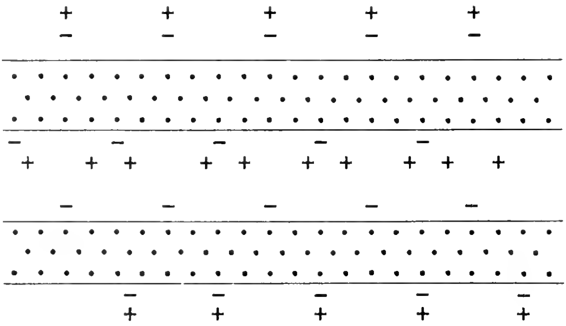


FIGURE 11–1. Charged surfaces of two lamellas of a clay particle.

ion. Na^+ and K^+ carry a large number of water molecules; Ca^{++} and Mg^{++} carry fewer. The H^+ ions also carry water as they do at all times, and in newer chemical nomenclature they are properly called hydronium ions. In addition to water of hydration carried by ions, water molecules are carried on the faces of these particles and by the spaces and channels between the thin sheets which make up the colloidal plates.

The composition of the clay colloidal materials varies greatly and depends upon such climatic conditions as temperature and rainfall during their formation, as well as on the composition of the parent rock. The ratio of silica to sesquioxides in soil colloids varies and seems to have an important effect on the properties of these particles. A soil containing a high ratio of silica to sesquioxides shows greater negative charge, dispersibility, viscosity, swelling, heat of wetting, adsorption of bases, and rate of base exchange. These colloids retain their negative charge in both acid and alkaline solutions. If a soil has a low silica-sesquioxide ratio the mineral colloids show an amphoteric behavior, for they become electropositive in acid solutions and will then absorb anions such as chloride ions and sulfate ions instead of positive ions.

Base exchange. The fixation of a positive ion by a colloidal particle of the soil is accompanied by the release of one or more previously held positive ions. Such an exchange of ions is commonly called base exchange or, more properly, cation exchange. An illustration of cation exchange is found in the gradual acidification of soil through the decomposition of organic matter and subsequent leaching of calcium. As organic matter decomposes in the soil, carbon dioxide is produced and combines with the soil water to form carbonic acid. The hydrogen ion of this acid will replace positive ions in the outer layer of the colloidal soil particles. If the particles contain a large amount of adsorbed calcium, the following change takes place:

$$Ca^{++}\boxed{\begin{array}{c}\text{Colloidal}\\ \\ \text{particle}\end{array}}^{=} + 2H_2CO_3 \rightarrow \begin{array}{c}H^+\\ \\ H^+\end{array}\boxed{\begin{array}{c}\text{Colloidal}\\ \\ \text{particle}\end{array}}^{=} + Ca(HCO_3)_2$$

Since calcium bicarbonate is soluble in water, leaching will remove this compound from the topsoil. The loss of calcium in this manner will gradually increase the acidity of the soil.

The following principles have been well established for base-exchange reactions of soils:

1. Adsorbed ions are replaced by other ions in equivalent amounts. Thus, if calcium replaces potassium, one calcium ion will replace two potassium ions.

2. Ions vary greatly in the ease with which they can be adsorbed or replaced by other ions. The ease or efficiency with which one ion replaces another ion is determined by its valence and its characteristic activity. Divalent ions are more potent than monovalent ions, with the exception of hydrogen ions. At equal ionic concentrations calcium and magnesium have greater replacing power than sodium and potassium. The activity of ions with the same valence also varies and seems to depend on the relative size of the hydrated ion. The smaller the hydrated ion, the greater its activity. When the following hydrated monovalent ions are placed in order of increasing size, we have Rb^+, NH_4^+, Na^+, Li^+. The decrease in replacing power of these ions follows the same order. If we compare the activity of these ions with the activity of some of the common divalent ions and with hydrogen ion, the descending order of replacing ability is found to be $H^+ > Sr^{++} > Ba^{++} > Ca^{++} > Mg^{++} > Rb^+ > K^+ > NH_4^+ > Na^+ > Li^+$. A comparison of the ease of adsorption of two of the ions in this series is shown in the work of Joseph and Oakley. They found that six times as many calcium ions as sodium ions were adsorbed when equimolar solutions of salts of these ions were allowed to percolate through certain soils.

3. Base-exchange reactions follow the law of mass action. An example with which the reader is familiar is found in the operation of the permutite water softener. Calcium and magnesium will replace sodium from the permutite as hard water is passed through the softener. But the reaction can be reversed and the permutite regenerated by forcing a saturated sodium chloride solution through it.

To soften water:

$$Na_2O \cdot Al_2O_3 \cdot 2SiO_2 + CaSO_4 \rightarrow CaO \cdot Al_2O_3 \cdot 2SiO_2 + Na_2SO_4$$

To regenerate the permutite:

$$CaO \cdot Al_2O_3 \cdot 2SiO_2 + 2NaCl \rightarrow Na_2O \cdot Al_2O_3 \cdot 2SiO_2 + CaCl_2$$

Another illustration of the operation of the law of mass action can be found in base-exchange reactions. If a substance is present that forms an insoluble compound with an adsorbed positive ion, such a cation will be replaced. For example, in the presence of phosphate and oxalate ions, calcium will be replaced by sodium and potassium, for calcium phosphate and oxalate are insoluble but the sodium and potassium compounds are soluble.

If a soil sample is treated with ammonium acetate solution, a reaction will take place in which NH_4^+ ions displace the previously adsorbed cations. The quantity of ammonium ion remaining in the soil after adequate washing may be determined and expressed as milliequivalents per 100 g. of soil. (A milliequivalent of an ion will combine with or replace 1 mg. of hydrogen.) The values so obtained are taken as a measure of the exchange capacity of a soil. Exchange capacity is another term for cation-adsorbing ability.

The exchange capacity of a soil varies with its composition. For example, the exchange capacity of clay is about 10 to 100 milliequivalents per 100 g. The importance of humus in the base-exchange complex is indicated by its high exchange capacity, 400 milliequivalents per 100 g.

Since the exchange of cations on soil colloids is called base exchange, the exchange of anions has been called acid exchange. However, acid exchange is not so well understood as base exchange. It is certain that most anions are not held by soil colloids as tightly as cations, for they are readily lost from the soil by moderate leaching. Nevertheless it is believed that anion as well as cation exchange does take place between the soil solution and soil colloids.

Soil solution. The components of soil, both organic and inorganic, appear to be difficultly soluble in water. However, soil compounds are soluble to a limited extent in the moisture which surrounds soil particles. The quantity of such dissolved material is always small at any one time, and the concentration of the solution varies greatly with the quantity of water present.

If soluble materials present in the soil solution are removed by plants or other agencies, the supply of plant nutrients is replenished by the dissolving of more material from the soil. Ions present in the soil solution are in equilibrium with ions adsorbed on the soil colloids. The maintenance of this ionic equilibrium by adsorption and release of nutrient ions is one of the important functions of soil colloids.

The soil solution of fertile soils contains a sufficient quantity of nutrients to supply the plants growing upon them. Excessive depletion because of overcropping or leaching leads to lower crop yields, and nutrients must be restored by the addition of manure or artificial fertilizer or by allowing the soil to lie fallow.

Absorption of plant nutrients. Our knowledge of the way in which nutrients are absorbed from the soil solution by plants is still fragmentary. Normal osmosis will account for the passage of water into root cells, for the soil solution is more dilute than the root cell solution. However, for the same reason the root cells should not be able to absorb molecules or ions from the more dilute soil solution. Nevertheless this anomalous movement of solutes into plants against a concentration gradient does take place. Such a phenomenon requires a source of energy.

The apparently abnormal behavior of soil ions in solution is known to have some connection with plant respiration. When plants die, the movement of solutes into plant cells ceases. Even living plants no longer absorb nutrients when placed in an atmosphere of nitrogen or carbon dioxide. Respiration must take place if plant root cells are to secure molecules and ions from the soil solution.

In our study of respiration and biological oxidation we have seen that the end products of this process are carbon dioxide, water, and energy. Carbon dioxide is given off by plant roots and is known to play an important part in plant-soil relationships. However, the principal effect of respiration on nutrient absorption may not be due to the action of carbon dioxide. It is more probable that the energy resulting from biological oxidation makes possible the anomalous diffusion of nutrients into root cells. Conceivably the energy produced may affect absorption by altering the charge on the semipermeable membranes of root cells.

The most important ions present in the soil solution are the following:

H_3O^+
NH_4^+
K^+
Ca^{++}
Mg^{++}
Cu^{++}
Zn^{++}
Fe^{+++}
Mn^{+++}

OH^-
NO_3^-
$H_2PO_4^-$
HCO_3^-
HPO_4^{--}
CO_3^{--}
SO_4^{--}
$H_2BO_3^-$
PO_4^{---}

Ions differ greatly in the rapidity with which they are absorbed by the growing plant. Of the common cations, potassium is most readily, and calcium least readily, absorbed. Of the common anions, the nitrate ion is absorbed most readily, and the sulfate ion appears to be least readily absorbed.

The absorption of a given ion by a plant is influenced by the presence of other ions. For example, the intake of potassium is more rapid when supplied as the nitrate than when supplied as the sulfate.

One step in the process by which plants obtain cations from the soil is believed to be an exchange, principally between the hydrogen ions from the plant roots and the cations from the soil complex. The absorption of cations is thought to take place in two ways. In the first way the exchangeable ion is absorbed from the soil solution, but in the second the absorption is thought to take place through contact exchange between plant roots and soil particles. This direct-contact exchange is believed to take place without having the ions become a part of the soil solution.

FERTILIZERS

A system of intensive agriculture which removes most of the plants from the farm on which they are produced sooner or later results in decreased crop yields unless certain practices in soil management are followed. In order to continue to produce good yields of crops, additional nutrients must be made available, either directly or indirectly.

Elements required for the maintenance of soil fertility are stored in soil minerals from which they may be released by growing plants. A system of rotation can be followed in which one crop is raised for the purpose of mining nutrient elements from soils. This plant crop when returned to the soil furnishes organic matter as well as available nutrients to help maintain soil fertility.

One of the best ways to make use of farm crops for the maintenance of soil fertility is to feed the plants to animals and to return the manure to the soil. The use of farm manure as a fertilizer and factors affecting its value will be discussed later in this chapter.

However beneficial the practice of returning plants to the soil may be, often it is not feasible to rely on this one method for the maintenance of soil fertility. Other sources of nutrient elements must be added. Materials that supply the lacking mineral elements are called fertilizers.

The usual meaning of the term *fertilizer* in commerce includes only the principal nutrient elements, nitrogen, phosphorus, and potassium, omitting calcium, magnesium, sulfur, and micronutrient elements. However, commercial fertilizers contain many elements in addition to the principal three given above. Thus sulfur, chlorine, sodium, calcium, magnesium, and small quantities of many other elements may be present. Nevertheless, fertilizers are sold on the basis of their content of nitrogen, phosphorus, and potassium. This content is stated as percentages of N, P_2O_5, and K_2O, given in that order. Therefore, a fertilizer the composition of which is given as 6–9–12 contains 6 per cent nitrogen, 9 per cent phosphorus calculated as P_2O_5, and 12 per cent potassium calculated as K_2O.

Because of the above conventions for indicating the analysis, it is customary to speak of fertilizer materials and mixtures as containing nitrogen, phosphoric acid, and potash. Until recent years it was also customary to speak of the ammonia instead of the nitrogen content of fertilizers. These terms have no significance with respect to the compounds in which the elements are found in fertilizers. Each of the three elements may be present in several different compounds.

Nitrogen Fertilizers

Four types of nitrogenous fertilizers are available. These are nitrates, ammonium compounds, organic compounds of nitrogen, and animal and vegetable residues.

Nitrates. The most common nitrate available for fertilizer is sodium nitrate (nitrate of soda). It occurs in a mixture of various salts, called caliche, found in large deposits in northern Chile. After purification by selective crystallization, the nitrate of soda is melted and sprayed into an enclosed chamber. The drops, on cooling, solidify into small shot-like pellets, a form that is convenient for packaging and handling. This pellet form contains about 99 per cent sodium nitrate.

Potassium nitrate is a desirable source of both nitrogen and potassium. However, the supply of nitrate of potash is quite limited. The principal source is caliche, which contains 2 to 3 per cent potassium nitrate. The commercial product derived from caliche is a crude grade which contains both sodium and potassium nitrate and has a nitrogen content of about 14 per cent and a potash content (K_2O) of about 15 per cent. Small amounts of purer KNO_3 are also available for fertilizer use.

Calcium nitrate, also known as nitrate of lime and Norwegian saltpeter, is an excellent source of quickly available nitrogen, containing about 16 per cent nitrogen and 34 per cent calcium, expressed as CaO. It is much more hygroscopic than either of the above nitrates, and because of this it is more difficult to apply to the soil. Owing to this fault it is not entirely satisfactory as a fertilizer, and its use has been limited. It is made in Norway and Germany by the neutralization of nitric acid with calcium carbonate.

Ammonium nitrate is also used as a nitrogenous fertilizer to some extent. Ammonium nitrate is a hazardous material, for it will ignite and detonate under certain conditions.

Nitrates are easily soluble in water, and this characteristic ensures a rapid availability of nitrogen for plant growth. Excessive quantities of nitrates are undesirable from the standpoint of permanent soil fertility because they tend to inhibit the processes of nitrification and nitrogen fixation. The residual effect of sodium, potassium, and calcium nitrates is to decrease soil acidity, whereas ammonium nitrate increases soil acidity.

Ammonium compounds. Of all the nitrogenous fertilizer materials, ammonium sulfate is the most widely produced and used. Much of it is prepared by the neutralization of dilute sulfuric acid with ammonia, a by-product of destructive distillation of coal. In the gypsum process, another common method for the preparation of ammonium sulfate, ammonia and carbon dioxide are passed into a suspension of calcium sulfate, forming ammonium sulfate and calcium carbonate.

$$2NH_3 + CaSO_4 + CO_2 + H_2O \rightarrow (NH_4)_2SO_4 + CaCO_3$$

Ammonium sulfate tends to increase soil acidity, and soils fertilized with this source of nitrogen must be limed more frequently than those treated with sodium nitrate. Aqueous solutions of ammonium sulfate are acid, since the compound is a salt of the weak base NH_4OH and the strong acid sulfuric. In addition soil acidity increases further as a result of nitrification. The reactions can be shown as follows:

$$NH_4^+ \rightarrow H^+ + NH_3 \rightarrow H^+ + NO_3^-$$

A popular method of explaining the acidifying effect of ammonium sulfate is to state that nitric and sulfuric acids are formed. Ammonium sulfate is a good source of nitrogen for most crops but not so satisfactory as nitrates for acid-sensitive plants such as beets, barley, and wheat. Other ammonium compounds sometimes used as nitrogenous fertilizers include ammonium chloride, ammonium nitrate, and several kinds of ammonium phosphates. Basic compounds such as lime or basic slag should not be mixed with ammonium salts, since a reaction takes place which liberates nitrogen as ammonia.

$$(NH_4)_2SO_4 + CaO \rightarrow CaSO_4 + 2NH_3 + H_2O$$

Anhydrous ammonia and various solutions with a high ammonium content are used as sources of nitrogen by the manufacturers of mixed fertilizers. These sources of ammonia have been derived either from the air by direct synthesis or from the by-products of other reactions. They represent the cheapest available source of nitrogen. Anhydrous ammonia or ammonia solutions are utilized by mixing them with superphosphate. A series of reactions takes place which results in the formation of monoammonium phosphate, ammonium sulfate, and several other compounds. Large quantities of ammonia are now being used by direct addition to irrigation water.

Organic nitrogen compounds. Urea is manufactured by combining ammonia with carbon dioxide under pressure. The reaction takes place as follows:

$$2NH_3 + CO_2 \rightarrow \underset{\substack{\text{ammonium}\\ \text{carbamate}}}{NH_2COONH_4} \rightarrow \underset{\text{urea}}{CO(NH_2)_2} + H_2O$$

Urea is readily soluble in water and is probably absorbed by plants in small amounts. But much of the urea is converted to ammonium compounds and nitrates in the soil and is absorbed as ions of these compounds.

$$\underset{\text{urea}}{CO(NH_2)_2} + 2H_2O \rightarrow \underset{\substack{\text{ammonium}\\ \text{carbonate}}}{(NH_4)_2CO_3}$$

$$\underset{\substack{\text{ammonium}\\ \text{carbonate}}}{(NH_4)_2CO_3} + 3O_2 \rightarrow \underset{\substack{\text{nitrous}\\ \text{acid}}}{2HNO_2} + 3H_2O + CO_2$$

$$\underset{\substack{\text{nitrous}\\ \text{acid}}}{2HNO_2} + O_2 \rightarrow \underset{\substack{\text{nitric}\\ \text{acid}}}{2HNO_3}$$

Urea is considered to be one of the best nitrogenous fertilizers because it is cheap, is readily soluble, is rapidly converted to ammonium and nitrate compounds, resists leaching, and increases soil acidity only slightly.

Calcium cyanamide, as produced for the fertilizer trade, is an impure, grayish black powder, containing a considerable amount of lime and free carbon. Pure calcium cyanamide ($CaCN_2$) is white and contains 35 per cent nitrogen. The commercial product contains about 21 per cent free CaO, 11 per cent free carbon, and smaller amounts of several other compounds.

The raw materials for the manufacture of calcium cyanamide are air, coal, and limestone. Nitrogen (prepared from air) and calcium carbide (produced from coke and lime) are heated at 1000°C. to form calcium cyanamide.

$$\underset{\substack{\text{calcium}\\ \text{carbide}}}{CaC_2} + N_2 \rightarrow \underset{\substack{\text{calcium}\\ \text{cyanamide}}}{CaCN_2} + C$$

Crude calcium cyanamide is particularly suitable for application to acid soil, owing to its high content of calcium oxide. When added to a soil having a pH of 7.0 or less, cyanamide is hydrolyzed to urea within a few days. It is this reaction which makes possible the use of cyanamide as a fertilizer, for the compound as such is toxic to plants. Plant toxicity is avoided if cyanamide is uniformly distributed and well mixed with the upper layer of soil and is applied at rates of 200 lb. per acre or less at least 10 days before planting.

Urea and calcium cyanamide are called "non-proteid organic fertilizers" by fertilizer manufacturers to distinguish these products from fertilizers of animal and vegetable origin.

Phosphate Fertilizers

The importance of phosphates is indicated by the fact, stated in a previous chapter, that low crop production is due more often to a lack of phosphorus than to a lack of any other element. All phosphatic fertilizers contain phosphorus as salts of *o*-phosphoric acid.

Rock phosphate. Rock phosphate, consisting largely of $Ca_3(PO_4)_2$, occurs in large deposits throughout the world. In the United States deposits of commercial importance occur in Florida, Tennessee, Idaho, Montana, Utah, and Wyoming. A limited amount of finely ground rock phosphate is used for direct application as a fertilizer. However, it is not a readily available source of phosphorus because the solubility of tricalcium phosphate is very low. Rock phosphate is not recommended when monocalcium phosphate, ammonium phosphate, or other more soluble forms can be obtained. The chief use of rock phosphate is in the preparation of other forms of phosphatic fertilizers.

Monocalcium phosphate. Monocalcium phosphate is one of the products formed when rock phosphate is treated with sulfuric acid.

$$1.\quad \underset{\text{tricalcium phosphate}}{Ca_3(PO_4)_2} + 3H_2SO_4 + 6H_2O \rightarrow \underset{\text{phosphoric acid}}{2H_3PO_4} + \underset{\text{calcium sulfate (gypsum)}}{3CaSO_4 \cdot 2H_2O}$$

$$2.\quad \underset{\text{tricalcium phosphate}}{Ca_3(PO_4)_2} + 4H_3PO_4 \rightarrow \underset{\text{monocalcium phosphate}}{3CaH_4(PO_4)_2}$$

A small amount of dicalcium phosphate, $Ca_2H_2(PO_4)_2$, is also formed during the reaction. The mixture of products formed from the above reactions contains about 50 per cent gypsum and 26 per cent monocalcium phosphate. This mixture is sold as the fertilizer called superphosphate or acid phosphate and contains 16 to 20 per cent available P_2O_5. An application of monocalcium phosphate may result in a temporary increase of soil acidity.

Concentrated superphosphate, called double or treble superphosphate, containing from 40 to 48 per cent available P_2O_5, is also manufactured. This product results when the calcium sulfate is removed in reaction 1 above and free phosphoric acid reacts with rock phosphate as in reaction 2. The mixture contains very little gypsum but may have a considerable quantity of free phosphoric acid.

We have already mentioned the fact that monammonium phosphate is produced when superphosphate is treated with anhydrous ammonia or ammonia solutions. The mixture of products obtained by this reaction is marketed as ammoniated superphosphate and contains from 16 to 18 per cent P_2O_5.

Basic slag, a by-product of the steel industry, is a relatively insoluble form of phosphatic fertilizer. But its phosphorus is far more available than that of rock phosphate. It contains from 8 to 25 per cent P_2O_5, as much as 50 per cent CaO, and varying amounts of Fe_2O_3, SiO_2, and other compounds. In terms of its principal constituents its composition is sometimes given as $P_2O_5 \cdot 4CaO$.

In the soil, basic slag is made available by the following reaction:

$$\underset{\substack{\text{basic}\\\text{slag}}}{P_2O_5 \cdot 4CaO} + 6CO_2 + 5H_2O \longrightarrow \underset{\substack{\text{monocalcium}\\\text{phosphate}}}{CaH_4(PO_4)_2} + \underset{\substack{\text{calcium}\\\text{bicarbonate}}}{3Ca(HCO_3)_2}$$

Bones are used as the parent material for several different products sold as phosphatic fertilizers. Raw bone meal, steamed bone meal, and precipitated bone are such products. The form of phosphorus present in bones is thought to be $Ca_3(PO_4)_2$. Dicalcium phosphate results when precipitated bone is made as a by-product of glue manufacture. Both raw and steamed bone meal contain nitrogen as well as phosphorus, but the cost of these elements per unit is higher when purchased in bone meal than when bought in other forms.

The term *available phosphorus,* or *available phosphoric acid,* as used in the fertilizer industry has a definite meaning. Available phosphorus is the sum of the water-soluble phosphorus and the ammonium citrate-soluble phosphorus. The value of available P_2O_5 obtained in this manner approximates the amount of phosphorus in a fertilizer which will be usable by plants.

Potash Fertilizers

Deposits of potassium salts are found in various parts of the world, but only those in France, Germany, and the United States are mined extensively. Sources of potash in the United States include the natural brines of inland lakes in Nebraska and California and deposits of sylvite (KCl) and kainite ($MgSO_4 \cdot KCl \cdot 3H_2O$) in New Mexico. Greensand, found in the eastern states, particularly New Jersey, is present in quantities sufficient to supply the potassium needs of the United States for hundreds of years. Many of these deposits are near or on the surface and can be mined by power shovels. Greensand is classified as glauconite ($KFeSi_2O_6 \cdot nH_2O$), the potassium of which is very slowly available. Its greatest value seems to be as a raw material for the production of K_2SO_4.

Potassium chloride (KCl) is the chief constituent of muriate of potash, manure salts, and kainite. The chloride is the most common form of potassium fertilizer and can be used on most crops. However, the quality of some plants is adversely affected by the application of large quantities of chlorides. The sugar content of beets is lowered, as is the quality of potatoes and the burning quality of cigar leaf tobacco, when KCl is used as a fertilizer.

Sulfate of potash and sulfate of potash-magnesia are marketed as potassium fertilizers. The sulfates are somewhat more expensive than the chlorides, and their use is limited to special-purpose fertilizers in which the chloride is undesirable. Sulfate of potash-magnesia is particularly desirable when magnesium is lacking in the soil. This double salt of potassium and magnesium contains about equal amounts of potassium sulfate and magnesium sulfate. Its K_2O content (about 25 per cent) is about one-half that of sulfate of potash.

Other potassium fertilizers include potassium nitrate, wood ashes, potassium phosphate, and tobacco stems. Sewage sludge and the various seed meals, such as cottonseed meal, also contain small and variable amounts of potassium.

Potassium salts used as fertilizers are water soluble and therefore should be readily available to plants. However, potassium salts added to certain soils with high clay contents appear to be changed to forms in which the potassium is as unavailable as that in soil minerals. This fixation of added potassium is a problem that has occupied the attention of investigators for many years. Experimental workers have secured good results from potassium fertilization by making one extremely large application of potassium salts to satisfy the fixing power of the soil. In subsequent years this is followed by small additions of potassium fertilizers for each crop.

Farm Manure

Farm manure is the solid and liquid excrement of animals, alone or mixed with litter. Litter is the straw, sawdust, or other absorbent material used in stables and barns. The term *manure* is sometimes applied to any fertilizer but is more often used, as it will be in this chapter, to mean farm manure. The value of manure for maintaining and improving soil productivity has been recognized from the earliest times. Its beneficial influence on soil fertility is much greater than can be accounted for by its content of the three principal fertilizer elements, nitrogen, phosphorus, and potassium.

Composition of farm manure. Fresh farm manure consists of solid and liquid components, the former approximating 75 per cent and the latter 25 per cent of the total weight. As a rule about one-half the nitrogen and potassium and nearly all the phosphorus are found in the solid portion. The feces may contain a considerable amount of undigested matter in the form of the original compounds of the ration. However, the urine does not contain undigested feed but contains waste products from the organs and tissues of the body. The compounds eaten by animals are hydrolyzed in the alimentary tract through the action of digestive or bacterial enzymes. As a result much of the fresh excrement consists of compounds less complex than the compounds ingested. The manure as voided contains numerous microbial species, including fungi, actinomycetes, and, particularly, bacteria. A relatively large part of the total weight of manure consists of the bodies of living and dead bacteria. The most resistant constituents of feedstuffs, from the standpoint of digestibility, are the various plant lignins, although considerable quantities of cellulose and hemicellulose also escape digestion. In the intestinal tracts of animals lignin combines with proteins (chiefly bacterial proteins) to form lignoprotein complexes, called humus. The humus of manure apparently is identical with the humus which is found in soils. It has been estimated that 25 per cent of the organic matter of cow manure is humus.

The composition of fresh or decomposed manure varies greatly and can be only approximately stated. Its content of N, P, and K is comparable to that of a low-analysis fertilizer and may not exceed 0.5 per cent N, 0.25 per cent P_2O_5, and 0.5 per cent K_2O. Only about one-half the nitrogen and potassium and about one-sixth the phosphorus are readily available to plants. Since the phosphorus is present in smallest amount and is less available than the other elements, it is evident that manure is not a balanced fertilizer and should be supplemented with a source of phosphorus. The amount of phosphate fertilizers that should be added to a ton of manure depends upon the acre-rate of application of the latter. If 10 tons is to be spread on an acre of land, each ton should be re-enforced with at least 50 lb. of 20 per cent superphosphate.

In addition to nitrogen, phosphorus, and potassium, manure contains substantial amounts of calcium, magnesium, and sulfur, and varying amounts of the micronutrient elements.

The factors that influence the composition of farm manure include kind of animal, age and ration of the animal, kind and quantity of litter, and storage conditions.

KIND OF ANIMAL. The nitrogen, phosphorus, and potassium contents of manure vary with the species of animal from which it is derived, as shown in Table 11–1. The composition of manure is affected not only by the species but also by the utility of the animal. For example, on maintenance rations steers may excrete in feces and urine a quantity of mineral nutrients equal to that contained in the feed ingested, whereas milch cows may utilize some of these elements for the production of milk. It has been estimated that, for each 1,000 lb. of milk, approximately 6 lb. of nitrogen, 2 lb. of phosphoric acid, and 2 lb. of potash are required.

TABLE 11–1. The Composition of Manure from Different Farm Animals as Based on 1-Ton Quantities

(Data of Duley, *Missouri Station Bulletin* 166)

Animal	Weight of Manure, lb.	Nitrogen, lb.	Phosphorus, lb.	Potassium, lb.
Horse				
Solid	1,632.2	8.06	2.12	3.26
Liquid	367.8	4.41	Trace	4.56
Total	2,000.0	12.47	2.12	7.82
Cow				
Solid	1,456.5	4.71	1.31	1.80
Liquid	543.5	5.16	0.06	4.29
Total	2,000.0	9.87	1.37	6.09
Sheep				
Solid	1,200.0	7.80	2.40	2.28
Liquid	800.0	13.44	0.10	14.08
Total	2,000.0	21.24	2.50	16.36
Hog				
Solid	1,290.3	7.74	2.58	4.77
Liquid	709.7	2.12	0.39	5.89
Total	2,000.0	9.86	2.97	10.66
Hen	2,000.0	23.00	8.10	7.46

AGE OF THE ANIMAL. Growing animals excrete less of the fertilizing constituents of their feed than do mature animals. A young growing animal requires considerable quantities of calcium and phosphorus as bone-building materials, and measurable quantities of these and other constituents are retained for the synthesis of body tissues.

RATION OF THE ANIMAL. The composition and quantity of a given animal manure vary with the kind, quantity, and digestibility of the feed consumed. For example, the digestibility of corn may exceed 90 per cent with the result that 10 per cent or less of the total quantity consumed may be recovered in the manure. If overripe timothy hay, which has a low degree of digestibility, is fed to animals, about two-thirds of the total dry matter ingested may be excreted.

KIND AND QUANTITY OF LITTER. The term *litter* is applied to material serving the double purpose of keeping animals clean and absorbing the water-soluble fertilizer constituents. Many different materials have been used for this purpose. These include cereal straws, cornstalks, hays, wood shavings, sawdust, peat, and other materials which have relatively high absorbent qualities. Wood products such as sawdust and shavings contain more lignins and resins than do straw, hay, and cornstalks. These compounds are resistant to the action of microorganisms, and, as a result, wood products are decomposed slowly. Straw, hay, and cornstalks contain a greater amount of readily decomposed carbohydrates, more protein, and more minerals than do wood products.

A substantial part of the components of a ration is changed by the animal into products which differ from the original compounds. Hence the composition of manure, as voided, differs from the composition of litter, although ration and litter may have a similar chemical analysis. Thus the ratio of litter to animal excreta will have an important effect on the composition of manure.

STORAGE OF MANURE. Conditions under which manure is stored have a profound influence on its composition. Loss of manurial constituents may take place through two principal agencies: (1) leaching, and (2) decomposition or fermentation.

Since a large proportion of the total nitrogen and potassium of farm manure is present in the form of soluble compounds, the possibility of their loss through leaching is usually great. An adequate amount of litter and tight floors prevent substantial losses from leaching in barns, but exposure of poorly built heaps to rainfall favors losses of soluble material. These losses are materially reduced if the heaps are built with steeply sloped sides and placed on concrete floors. Storage of manure in a roofed concrete pit will further reduce losses from leaching.

Not only does manure contain relatively large quantities of soluble compounds, but also other materials are present which can be changed to soluble or volatile compounds as a result of decomposition or fermentation. Decomposition of stored manure is a continuous process, the rate of which varies according to environmental conditions.

Decomposition of manure. The liquid portion of manure is relatively rich in nitrogen, chiefly in the form of urea ($CO(NH_2)_2$). Through the activities of microorganisms, urea is broken down to ammonia and carbonic acid, which may unite to form ammonium carbonate ($(NH_4)_2CO_3$) or the acid carbonate (NH_4HCO_3). Both compounds are unstable, their instability being increased by increased temperatures, alkalinity, and drying. When ammonia evolves in the presence of a limited amount of carbon dioxide, the reaction of the decomposing material may reach pH 9.5. A slightly acid reaction, equal to pH 6.6, practically inhibits decomposition of the urea.

In addition to the readily decomposable urea, manure contains proteins. Some of these come from the feed or are present in the litter; others are of bacterial origin. Proteins undergo hydrolysis with the liberation of amino acids, which, through biological oxidation, yield nitrogen in the form of ammonia or elemental nitrogen. Numerous other compounds arise as a result of protein degradation. These include indole, skatole, mercaptans, hydrogen sulfide, and amines, in addition to various organic acids.

The manurial solids contain considerable quantities of lignin, cellulose, hemicellulose, and related compounds. The rate and course of decomposition of the carbohydrates and related compounds vary with the environmental conditions. Decomposition is extremely rapid at relatively high temperatures with sufficient air supply and adequate moisture. An excess of moisture, through its interference with aeration, limits the reaction.

When anaerobic rather than aerobic conditions prevail, there is an accumulation of intermediate compounds resulting from the decomposition of carbohydrates, proteins, and related compounds which include various organic acids, ammonia, hydrogen, methane, and carbon dioxide. The acids tend to combine with ammonia, forming ammonium salts, and thus reduce the loss of nitrogen through the volatilization of ammonia. At the same time the pH of the manure is lowered.

During the decomposition of manure there is a simplification of numerous compounds and the synthesis of others. The final result of the synthesis of organic compounds is the formation of humus, a complex so resistant to decay that it may be considered almost stable.

Chemical treatment of manure. Concern over the rapid loss of ammonia from manure has led to the use of chemical preservatives.

For a long period of time gypsum has been employed for this purpose. The reaction results in the production of calcium carbonate and ammonium sulfate.

$$CaSO_4 + (NH_4)_2CO_3 \rightleftarrows CaCO_3 + (NH_4)_2SO_4$$

Superphosphate, which contains both gypsum and monocalcium phosphate, functions better than either of these compounds alone, the reaction taking place according to the equation:

$$2CaSO_4 + CaH_4(PO_4)_2 + 2(NH_4)_2CO_3 \rightarrow Ca_3(PO_4)_2 + 2(NH_4)_2SO_4 + 2H_2O + 2CO_2$$

Tricalcium phosphate, unlike calcium carbonate, does not react with $(NH_4)_2SO_4$, and the above reaction is not reversible. Thus the addition of superphosphate accomplishes two results: it serves as a preservative for ammonia, and it supplies phosphorus to create a better-balanced fertilizer.

Many materials having bactericidal properties have been employed as preservatives. These include formalin, chloropicrin, and the sulfates of iron, zinc, and copper. Strong acids, such as sulfuric, phosphoric, and hydrochloric, have been used to a certain extent. The latter compounds prevent the loss of ammonia through the formation of ammonium salts and change the pH to a value which inhibits fermentation. Whether the retention of ammonia compensates for the inhibition of bacterial life is open to question.

Effects of manure on soil. The beneficial effect of an application of manure as reflected by plant response may be very great. This effect may be apparent for only 1 year when manure is applied to gravelly or sandy soils, but when it is applied to heavier soils profitable crop increases may be noted from 2 to 4 years later. At the Rothamsted Station the effects of eight yearly applications of 14 tons each were apparent 40 years after the last treatment.

CHEMICAL EFFECTS. Additions of manure to a soil increase the quantity of mineral nutrients available for plant growth. The increase of available nutrients results in part from the liberation of plant nutrients carried by the manure and in part from materials made available from insoluble soil minerals. This is true especially for calcium, magnesium, potassium, manganese, and to a certain extent for other elements. The exchange capacity of manured soils is considerably increased over that of non-manured soils, reflecting the effect of added humus.

PHYSICAL EFFECTS. When manure undergoes a rapid decomposition in heavy soils, it improves the structure, allowing better aeration. The improvement in structure favors root development and plant growth and decreases soil erosion. Applications of well-decomposed manure improve light sandy soils by increasing their moisture-holding capacity.

The greater humus content following manurial treatment darkens the soil and increases heat absorption from the sun. The higher temperature of the soil is advantageous where early development or growth is desired.

BIOLOGICAL EFFECTS. The biological effects of a manure treatment may be more important than the physical and chemical effects. An application of manure supplies numerous bacteria and other organisms, a number of which will continue to thrive and to multiply. Such an addition stimulates the multiplication of many native soil organisms as well. The metabolism of soil organisms produces cementing substances called "bacterial cement," which causes aggregation of soil particles.

The fact that small applications of manure exert a much more favorable influence on plant growth than can result from the nutrient content alone has been attributed to the inoculation of the soil with desirable organisms and to the increased development of similar organisms peculiar to the soil. This increased bacterial activity is thought to result in the production of plant stimulants analogous to vitamins and hormones.

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Growth regulation

12

In preceding chapters the principal concepts of plant metabolism, seed germination, and plant nutrition were introduced. In each instance growth was the ultimate result of the various physical and chemical processes described. As pointed out in Chapter 1, growth of a living organism results from a synthetic development from within. Such growth, which implies both enlargement and differentiation, is not haphazard but rather is controlled by several "built-in" series of interrelated factors. In plants, two such series include the genetic or hereditary factors and hormonal or growth-correlating factors, the latter probably being largely dependent on the former. A third group includes the vast number of environmental factors which contribute greatly to the growth of the plant in question. While it is beyond the scope of this book to treat the genetic factors, it is appropriate to discuss the last two groups in some detail.

ENVIRONMENTAL FACTORS INFLUENCING GROWTH

Environmental factors influencing the growth of plants can be grouped into three classes, climatic, edaphic, and biotic. Climatic factors influence the growth of a plant through the atmosphere and include such things as temperature, light, carbon dioxide concentration, precipitation and humidity, and wind movement. Edaphic factors operate through the soil and include soil moisture, temperature, aeration, and solute concentration. Biotic factors arise from the presence of other plants and animals. In this group are included such items as species competition, animal grazing, soil bacteria, fungi, and insects. A number of the environmental factors have been considered in detail in other chapters or are obvious in their effect on plant growth. The following discussion is limited therefore to a few of the other environmental factors relating to plant growth.

Temperature

All chemical, physical, and physiological processes in a plant are directly affected by temperature. Such processes, in turn, govern both character and rate of plant growth. Since each process can be considered critical and limiting, modification of any one of them may affect growth of a particular part of the plant or the overall growth of the whole plant.

Most of the reactions taking place in a plant are enzyme-catalyzed. A rise in temperature increases the velocity of such reactions, but only within definite limits. Since an enzyme is heat sensitive because of its protein nature, two processes are affected by a rise in temperature: (1) an increase in the velocity of the catalyzed reaction due to greater molecular activity, and (2) an increase in the rate of destruction of the enzyme by heat denaturization. Therefore, the temperature at which maximum velocity takes place is not necessarily the optimum temperature for enzyme action, since this rapid velocity can be maintained for only a short time. The optimum range of temperatures for many plant enzymes lies between 70 and 80°F.

Temperature is an important factor in initiating flowering in plants. In the case of beets, radishes, and carrots, of bulbs such as tulips and hyacinths, of trees such as apple and peach, and of cereal crops such as winter rye and winter wheat, low temperatures are particularly important for flowering. The induction of flowering as a result of low temperatures is known as vernalization.

Light

When referring to light as an environmental factor influencing plant growth, one includes all radiant energy that reaches the plant from the sun or from artificial sources. Light affects plant growth in many ways, including among others the processes associated with photosynthesis, the temperature of that portion of the plant above ground, the direction of growth of certain plant organs, and the form and anatomy of the leaves produced. Thus it is evident that light intensity, quality, and duration play important parts in influencing plant growth.

Some plants flourish in dense shade (light of low intensity), whereas others grow normally under conditions of high light intensity. Most plants, on the other hand, show growth in complete darkness just as long as they have a reserve food supply. Contrasted to green plants growing in the presence of light, those grown in darkness have characteristically small leaves which are white or yellow in color, and poorly developed root systems. Such plants, said to be in a state of etiolation, cease to grow as soon as the reserve food supply is exhausted. In most cases, the absence of light increases and the presence of light decreases the rate of stem elongation. Plants which have the benefit of full light produce shorter, thicker stems than plants grown under low-intensity light. Thus, plants that are crowded have a tendency to be spindling and consequently more fragile than those which have been given ample room for development.

The effect of different wave lengths of light on the photosynthetic process was discussed in Chapter 9. Some general conclusions can be stated with regard to light quality and plant growth. Thus, light of short wave length (blue region) retards growth in length, whereas long wave lengths (red region) have a tendency to produce spindling effects.

Many plants react quite characteristically when exposed to varying periods of light and dark. The response of plants to the relative length of light and dark periods is called photoperiodism. Thus, it has been found that certain plants do not flower until the photoperiod reaches a certain critical value for an extended period of time. For instance, spinach does not form flowers until the day length reaches at least 13 hours for a period of not less than 2 weeks. Plants that react in this way to long photoperiods are said to be long-day plants and include henbane, spinach, dill, plantain, sugar beet, and winter barley.

Other plant species, such as *Chrysanthemum*, flower only when the daily period of illumination is shorter than a certain critical value. Such plants are called short-day plants. Thus, chrysanthemums remain vegetative if the period of exposure to light exceeds 14 hours and produce flowers only when the day length is less than this critical value. Such plants as chrysanthemum, ragweed, poinsettia, and cocklebur are all dependent on long periods of darkness.

Finally, there are certain species of plants such as tomato, snapdragon, and buckwheat that bloom throughout the growing season. Such plants are indifferent to the relative lengths of day and night.

In addition to flowering, the photoperiod is known to affect many other plant characteristics, among which are leaf shape, leaf thickness, the bulbing of onions, and the tuberization of potatoes.

Biotic Factors

As described earlier, biotic factors are those which connote competitive growth on the part of other plants and animals in close proximity to the one of immediate interest. Thus, living organisms surrounding a particular plant form as much a part of its environment as do temperature, light, mineral salts in the soil, and other climatic and edaphic factors. In fact, biotic factors influence the growth behavior of the plant throughout its life. As might be expected, some factors favor plant growth whereas others are distinctly inhibitive to such growth.

Plants which are crowded close together are forced to compete with each other for space, light, water, and mineral salts in the soil. Thus there is competition both above and below ground. Such overcrowding obviously results from failure to thin plants of the same variety or failure to remove undesirable plants (weeds) of other varieties.

Whereas many plants depend upon insects to effect pollination, these same plants may be destroyed by the ravages of other insects, bacteria, or fungi. For example, certain bacteria, fungi, nematode worms, and insects live parasitically on the roots of plants. Moreover, many different kinds of insects, fungi, viruses, and bacteria attack the aerial portions of a plant, causing destructive diseases.

Soil fertility is also influenced by the activity of certain fungi and bacteria which cause decomposition of organic matter into materials available to green plants. Some soil bacteria also bring about nitrogen fixation. Certain soil fungi influence the growth of some plants, particularly forest trees. In this instance, the soil fungi attach themselves to the roots of the trees, sometimes even penetrating the living root cells. The presence of such a fungus is quite often beneficial in that the parasite makes readily available to the host some of the nitrogenous compounds of the soil. Such fungi are also beneficial in that they facilitate absorption and transfer of minerals from the soil to the host.

Finally, grazing animals contribute greatly to the alteration of vegetative growth in a particular area. This effect may vary from selective removal of certain plants (thus encouraging growth of others) to complete overgrazing by certain animals with the ultimate destruction of rangeland. With regard to the latter, it is well known that the perennial grasses of rangeland of our western states have been seriously depleted as a result of excessive and improper grazing. Rejuvenation of such grassland is a time-consuming and costly operation.

PLANT HORMONES AND REGULATORS

All multicellular organisms are integrated so that the various parts function together as a closely coordinated unit. By careful regulation of growth, development, and function, an organism is produced which is always characteristic of the species. Moreover this organism is capable of responding to external stimuli and therefore is sensitive to its environment. In plants this response is accomplished without benefit of a nervous system and specific sense organs, such as occur in most animals.

Plants, like animals (page 312), have hormones that are produced in one part of the organism and that influence a specific physiological process when transported to another part of the organism. Contrary to animals, plants do not produce hormones in any specific glands, but can manufacture them in a variety of plant tissues. Plant hormones also differ greatly in chemical structure from those of animal origin.

Substances which act like hormones but are not known to be produced by the plant are called plant regulators. The study of plant hormones and regulators is one of the most interesting fields of plant physiological research, and their use in agriculture has become very important.

Growth Hormones

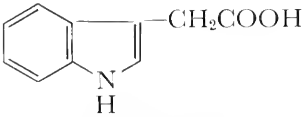
The first postulation of the existence of a plant-growth hormone was advanced by Charles Darwin in 1881. As a result of some simple experiments using canary-grass seedlings, a light source, and a razor blade, Darwin concluded that the tip of the grass shoot governs the phototropic response. Excision of the tip of the sheath-like structure which encloses young leaves of grasses (coleoptile) resulted in the loss of sensitivity of the remaining portion to unilateral light. When the tips of intact coleoptiles were covered with little tin-foil caps, a similar loss of sensitivity to light occurred. When the lower part of the coleoptile was wrapped with tin foil and only the tip was exposed to unilateral light, the tip bent towards the light. From these findings Darwin concluded that "when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper to lower part, causing the latter to bend."

In 1919 Paál discovered that, if he cut off the tip of an oat coleoptile and applied it against the side of the cut stump, the coleoptile was induced to grow on that side and to bend away from the tip. From this he concluded that the curvature was due to an unequal distribution of a growth-regulating substance, and that the tip of the coleoptile is the source of this substance.

In 1928 F. W. Went at Utrecht came to the conclusion that a growth-hormone system is essential to all plant growth. He demonstrated that the growth hormone can be isolated by diffusion of the substance into an agar block. If the agar block containing the hormone was pressed against the side of a coleoptile stump, the coleoptile bent away from the block. When the hormone from more than one tip was transferred to an agar block, Went found that the degree of bending was proportional to the number of tips and hence to the amount of hormone in the block. Thus, Went provided a means of measuring the concentration of the hormone and a method for the isolation of the active substance.

When the amount of a biologically active substance is measured by application to or injection into a living organism, the technique is called a bioassay. This method is used frequently to evaluate quantitatively very small amounts of substances, although refined chemical techniques rapidly are replacing the bioassay as increased information allows their development.

The principal growth substance isolated from higher plants is 3-indoleacetic acid. It has been isolated from a large number of monocot and dicot species and has been identified in certain fungi. Indoleacetic acid is produced in greatest concentration in the apex of the shoot and in the tip of the root, although it is also found in other actively dividing tissues, such as expanding leaves, flowers, and fruits. As the leaf, flower, or fruit matures, the amount of growth hormone produced decreases materially, although it never completely disappears. In fact, the formation of growth hormones by mature leaves seems to indicate that growth is not the sole prerequisite of hormone production, since a sizable quantity of the growth substance is formed even when there is little change in size.



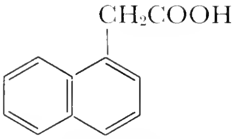
3-indoleacetic acid

The movement of the growth hormone in a plant is from the tip of the shoot to the older portions of the plant. So powerful is the movement of the substance towards older tissue that if a section of the tip is removed and inverted, the growth hormone is transported from the apical portion to the original basal portion but not in the other direction.

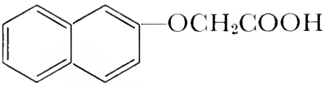
In addition to indoleacetic acid several other compounds have been isolated from plants which seem to have growth-promoting properties. Indolepyruvic acid has been identified in plants by chromatographic techniques. Although this compound is known to have growth-promoting activity, the fact that it is readily broken down to indoleacetic acid makes it difficult to assay its role as a growth hormone.

Three other compounds possessing growth-stimulating properties have been identified in plants, although their role as growth hormones is obscure. These substances are *cis*-cinnamic acid, phenylacetic acid, and indoleacetonitrile. In all probability there are still other growth hormones not yet identified, although it seems quite clear that indoleacetic acid is by far the most common.

A large number of synthetic compounds with growth-stimulating properties similar to indoleacetic acid have been produced. These synthetic compounds, together with the naturally occurring growth substances, have been given the general name *auxin*. Certain naphthalene derivatives were among the first synthetic compounds to be examined for auxin activity. 1-Naphthaleneacetic acid is widely used as an overall growth-stimulating substance. It is particularly useful in the rooting of cuttings and in the treatment of seeds before germination. 2-Naphthoxyacetic acid is particularly useful in setting of seedless fruit (parthenocarpy) by the application of this substance to the pistils of various flowers.



1-naphthaleneacetic acid
(NAA)



2-naphthoxyacetic acid
(NOA)

In addition to those organic compounds containing naphthalene or indole rings, certain synthetic benzene derivatives produce growth responses in plants. Both phenylacetic and phenoxyacetic acids have some activity, the latter only very weakly. Whereas phenylacetic acid is useful in setting of certain fruits, phenoxyacetic acid finds its greatest importance as the parent compound of a number of important derivatives, including 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). These substances are among those widely used growth regulators classed as herbicides.

Antiauxins

A variety of substances are capable of inhibiting growth even in the presence of auxins. Such compounds, called antiauxins, serve to reduce the effectiveness of auxins, though it is not clear whether they directly antagonize the action of the auxin or whether they inhibit growth by an indirect means.

Maleic hydrazide, an example of an antiauxin, is capable of inhibiting plant growth without causing any obvious morphological abnormalities. It appears to produce effects opposite to those induced by indoleacetic acid. Thus, maleic hydrazide inhibits terminal growth and stem elongation and destroys apical dominance. Recent experiments indicate that the inhibitory effects displayed by this compound can be neutralized by the addition of more auxin. Thus it is believed that the original growth inhibition results when maleic hydrazide stimulates an enzymatic destruction of indoleacetic acid.

Although maleic hydrazide inhibits growth (auxin) activity, it stimulates maturation processes such as production of lateral buds and initiation of leaf and fruit abscission. Apparently, then, maleic hydrazide is a true growth-inhibiting and maturity-inducing substance.

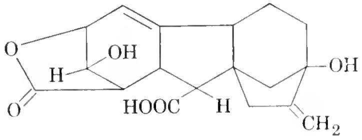
Flower-forming Hormones

The economic importance of controlling flower formation must not be underestimated. For example, in order for crops such as celery, spinach, cabbage, lettuce, beets, and carrots to have market acceptance, they must remain in the vegetative state. On the other hand, it is equally important that certain other plants be encouraged to flower and to produce fruits and seeds. Included in this category are the cereals, various fruits, vegetables, and ornamentals.

At the time that plants change from vegetative to reproductive development, the naturally occurring growth regulators, such as 3-indoleacetic acid, reach a low level in the young active regions of the plant. From this behavior it seems reasonable that external application of growth regulators could delay or inhibit flower formation. In actual practice, flowering has been delayed or entirely prevented in petunias, snapdragons, larkspurs, salvia, stocks, and iris following application of 3-indoleacetic acid. Thus growth regulators are used to supplement temperature and day-length adjustments in regulating flowering in many floricultural crops.

As described in a previous section, flowering depends to a large extent on the exposure of a plant to alternate periods of light and darkness, and seems to be controlled by hormonal substances called "florigens" which induce flower formation. Although all attempts to isolate these florigens have been unsuccessful, many workers feel that the flowering hormones are produced in the leaves and are translocated to the growing points. Moreover, it is generally agreed that these hormones are not species specific but rather that the same substances are active in long-day and short-day plants.

The fungus *Gibberella fujikuroi* produces the substances gibberellic acid, gibberellin A_1, and gibberelin A_2, which are active in promoting shoot growth by cell elongation and, in addition, stimulate flower formation. Although the chemical formulas for gibberellins A_1 and A_2 have not been conclusively demonstrated, gibberellic acid appears to be a tetracyclic dihydroxylactonic acid with the structural formula



gibberellic acid

Root-forming Hormones

The portion of a plant that is severed from the parent plant for the purpose of propagation is called a cutting. Thus the formation of roots on such cuttings is an important method of plant propagation.

Shortly after 3-indoleacetic acid was recognized as a plant-growth regulator, it was discovered that this same substance promoted root formation when applied to stems and cuttings of some plants. Today such closely related compounds as 3-indolebutyric acid (IBA) and 1-naphthaleneacetic acid (NAA) are commonly used in horticultural practice. The amide derivative of naphthaleneacetic acid, naphthalene acetamide (NAd), is also in common use. The potassium salts of IBA and NAA, as well as the methyl esters of these compounds, are the most effective forms, since they have the advantage of sufficient solubility in water.

2,4-Dichlorophenoxyacetic acid (2,4-D) promotes root growth at low concentrations but at the same time inhibits bud growth. Trichlorophenoxyacetic acid (TCP) does not inhibit bud growth and therefore is generally more suitable for rooting practices. 2,4-Dichlorophenoxypropionic acid and 2,4-dichlorophenoxybutyric acid are also quite effective substances in promoting root formation and growth. Owing to the relatively low cost of production, the last four compounds are used in large-scale plant-propagation operations.

There are a large number of ways to introduce the root-growth stimulator onto or into the cutting. While any of these are satisfactory, those which are most convenient include the application of powder mixtures to the bases of cuttings, quick dipping of the bases into concentrated solutions, and prolonged soaking of the bases in dilute aqueous solutions. Each of these methods has certain advantages and disadvantages, and no single method of application has clear superiority over the others. Some plants respond better to one method than to another.

A number of conditioning factors play an important role in the response of a cutting to the administration of a rooting hormone. For example, it is possible that a deficiency of food materials might become a limiting factor in the production of roots on a fresh cutting. For this reason, sucrose is generally incorporated in the dilute aqueous solutions of the growth regulators. The addition of sugar is quite helpful with difficult cuttings and increases the root mass on most cuttings. When sugar is used in the rooting solution, care should be taken to wash the excess sugar solution from the surface of the cutting in order to reduce the possibility of fungal growth.

Substances containing nitrogen, for example amino acids, ammonium sulfate, and the like, often are beneficial additions to the rooting solution. They are particularly useful in instances where cuttings produce roots only with great difficulty.

Like the nitrogen compounds, certain vitamins are thought to aid root formation. Favorable responses have been observed when thiamine, nicotinic acid, and ascorbic acid have been added to the root growth-regulator solution.

Inhibition of Sprouting

Each year the loss of millions of bushels of potatoes, carrots, beets, turnips, and onions from sprouting during storage is of significant economic importance. Although much sprouting can be controlled by low-temperature storage (below 40°F.), such storage is expensive, and, if accomplished, the end results are not always the most desirable. For example, potato storage at temperatures lower than 40°F. results in an accumulation of reducing sugars such as glucose. To the manufacturer of potato chips and dehydrated potatoes this is undesirable, since these sugars are partially responsible for an undesirable browning of potato chips and an objectionable discoloration of dehydrated potatoes.

Although sprouting of potatoes soaked in a solution of 3-indoleacetic acid is somewhat suppressed, the use of this chemical is not very feasible owing to the fact that its entry into a whole, uninjured tuber is very slow. On the other hand, the methyl ester of 1-naphthaleneacetic acid (MENA) is used extensively since it is quite volatile, easily applied in vapor form, and penetrates the tuber readily. Exposure of potato tubers to vapors of MENA effectively prevents sprouting for 1 year when the tubers are stored at temperatures between 50 and 60°F. In fact, sprout inhibition occurs at temperatures as high as 75°F. for several months, although storage at this temperature results in some shriveling. Although only very small amounts of MENA are retained by the potato and most of this is in the skin, germination of treated tubers is severely retarded. Most of the retarding effect can be overcome if the potatoes are washed with soap and water before planting.

A considerable amount of work has been done relative to the application of sprout-inhibiting substances directly to the plant in the field. Foliar sprays of 2,4-D, MENA, IA, and NAA have all resulted in significant decreases in the sprout growth of tubers which were subsequently stored at 50°F. Foliar applications of maleic hydrazide prevent sprouting of potatoes subsequently stored for 8 months at 57°F. In the latter case, there is an increased sucrose content and a reduced loss of sucrose during storage. Both cooking quality and color of potato chips have been improved by storage of the potatoes at such a relatively high temperature.

Although the prevention of sprouting in onions by direct application of growth regulators to the harvested bulbs has met with little or no success, foliar sprays, prior to harvest, are quite promising. In fact, marked success is reported from foliar sprays of maleic hydrazide applied 2 weeks before harvesting. Under such conditions, onions have been stored in perfect condition for at least 7 months.

Root crops such as carrots, beets, turnips, and rutabagas respond to postharvest treatment with MENA and NAA. Foliar application of maleic hydrazide several days before harvest inhibits postharvest sprouting of carrots, beets, parsnips, rutabagas, and turnips without any reduction in crop yield.

Abscission and Plant Regulators

Abscission is the term used to describe the separation of an organ or other plant part from the plant body. The dropping of fruit and autumnal leaf-fall are classical examples of this process. Under normal conditions abscission may be considered as a final process in a cycle of growth processes. In fact, abscission serves a number of useful functions in the survival of an individual plant or in the perpetuation of the species. Among these functions should be mentioned the dispersal of seeds, fruits, and other reproductive structures, the shedding of leaves under drought conditions in order to reduce rate of water loss, the dropping of excess young fruits to permit the remaining fruits to attain greater size and retention to maturity, and the shedding of bark, leaves, and other portions as a part of the regular excretion process. An impressive amount of evidence points to the fact that abscission of an organ is inhibited so long as its auxin concentration remains high, but that the abscission process is initiated as soon as the auxin level decreases materially.

The regulation of the abscission process has attained great commercial significance to producers of agricultural products. Whereas in some instances it is desirable to delay the process, in others it is important that abscission processes be accelerated. Following are several examples to illustrate the significance of regulation of this natural process.

A number of growth regulators have proved valuable in inhibiting or retarding leaf-fall. For example, 2,4-D applied as a preharvest spray inhibits the defoliation of cabbage and cauliflower during storage. In a similar fashion the inclusion of 2,4-D in the oil sprays required for the control of red scale on citrus largely prevents the defoliation which follows the use of the oil sprays alone. In holly, defoliation is often induced by the high humidities and traces of ethylene which are frequently encountered during the shipment of the refrigerated product. Such defoliation can be completely avoided by spraying the plants with NAA before shipment.

In the Northern Hemisphere during the month of June a considerable amount of fruit is lost owing to the process of premature abscission. In a number of instances, June drop can be controlled by the judicious use of certain plant regulators. For example, the addition of small quantities of NAA to sprays of lead arsenate or dispersible sulfur greatly reduced June drop of a number of varieties of apples. In other instances the addition of 2,4-D to the spray had the same desirable effect.

In some cases, controlled defoliation of a plant is desirable. For instance, the removal of leaves from the cotton plant before harvesting the cotton is desirable to prevent staining of the fiber. Moreover, such defoliation reduces the possibility of insect damage and boll rot. In general, monosodium cyanamid and calcium cyanamid are most effective for this purpose. These compounds are also used to bring on defoliation of young nursery stock before digging and storing. In this case, the removal of leaves is an important step in preventing the plants from becoming infected during storage.

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ANIMAL BIOCHEMISTRY

part 3

Body tissues

13

BLOOD AND LYMPH

Blood

Blood is a circulating tissue that is peculiar to the higher animal world. Unicellular animals and plants absorb their food from the surrounding medium and excrete waste products into the same medium. Multicellular organisms are not able to function in this simplified manner since most of the cells are buried beyond the limits of active or passive diffusion from the surrounding medium. In such animals the transport of food to, and waste from, the cells is accomplished by the flow of body fluids such as blood. Although blood is a living tissue, the rate of metabolism is rather small compared with that of liver and other metabolically active components of the body.

The principal functions of blood are (1) to carry nutrients from the digestive tract to the body tissues, (2) to transport waste products of metabolism to the excretory sites, (3) to transfer oxygen and carbon dioxide between the lungs and tissues, and (4) to distribute hormones, immune substances, etc., to the various parts of the body. In addition to these transport and distribution functions, blood also plays important roles in pH and osmotic pressure maintenance and in the distribution and radiation of heat.

When blood is subjected to low-speed centrifugation, slightly more than 40 volume per cent separates as solids or corpuscles. The remaining 60 per cent consists of a straw-colored fluid called plasma. The corpuscular fraction consists almost wholly of red blood cells, the erythrocytes. The other solid constituents, the white blood cells (leucocytes) and the blood platelets, constitute only about 0.2 per cent of the volume of the corpuscles.

The average specific gravity of normal human blood varies from 1.055 to 1.060, although in abnormal situations specific gravities from 1.035 to 1.075 have been observed. There is a tendency for the specific gravity of blood to be lower during sleep than during waking hours, and it is increased by exercise and decreased slightly after meals. There is so much variation between individuals that a value normal for one person may be an index of a pathological condition in another. The corpuscles have a higher specific gravity than the plasma, and erythrocytes are heavier than leucocytes.

The pH of blood is approximately 7.4 with an extreme normal range of 7.3 to 7.5. This remarkably uniform pH is the result of the buffering capacity contributed by hemoglobins, plasma proteins, phosphate ions, and bicarbonate ions. Bicarbonate ion concentration is controlled through carbon dioxide excretion from the lungs at a rate proportional to its concentration in the blood.

The osmotic pressure of blood is also kept very nearly constant in spite of continual exchange of nutrients, waste products, and water on the circuitous passage through the tissues. The rate of water absorption from the intestine and the rate of loss through the skin and the lungs are dependent on external conditions. The kidneys serve an important function in controlling the water and salt content and, therefore, the osmotic pressure of the blood. The osmotic pressure of the human blood is equivalent to that of a 0.9 per cent solution of NaCl. The latter is known as physiological saline solution and is sometimes used for intravenous injections. Ringer's solution is preferred to physiological saline because it contains a better mixture of cations. Ringer's solution for mammals consists of NaCl (0.90 per cent), $CaCl_2$ (0.26 per cent), and KCl (0.03 per cent).

On the basis of body weight, the amount of blood in man varies from 6 to 8 per cent for men and 5 to 7 per cent for women. Blood accounts for 2.5 to 5.0 per cent of the body weight of swine, 7.7 to 10.0 per cent for chickens, and 10 per cent for horses. The blood volume increases in the summer time and decreases in winter because of surface capillary constriction in colder weather.

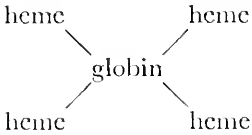
Erythrocytes. The number of erythrocytes per unit volume varies in different animals. In man the average number is about 5,000,000/cu. mm., whereas the blood of women averages about 4,500,000. Individuals fully acclimated to higher altitudes show a higher red cell count and an increased hemoglobin content. The erythrocytes contain about 30 per cent hemoglobin, 3 per cent stroma protein, lipides, and inorganic ions, and 67 per cent water. When the erythrocytes are ruptured, or if the permeability of the cell membranes is increased, hemoglobin escapes and forms a homogeneous solution. Such blood is said to be laked or hemolyzed.

Erythrocytes are produced in the bone marrow and to a limited extent in the spleen by a process called hemopoiesis. Nucleated daughter cells are formed as buds on the large erythroblasts of the bone marrow. After the cells are budded off they enter the general circulation, gradually lose their nucleus, and accumulate hemoglobin. The non-nucleated erythrocytes do not have a long life, estimates of the average life span varying from 20 days to 125 days. Continuous disintegration of aged erythrocytes occurs in the blood vessel walls of the liver, giving rise to the bile pigments (page 470).

The primary function of the erythrocytes is the transference of oxygen from the lungs to rapidly metabolizing tissues. Hemoglobin is the component of the red cell that is directly involved in this transfer.

Hemoglobin is a conjugated protein consisting of the iron-containing pigment heme and the protein globin, a member of the histone (page 114) class. The hemoglobin content of normal human blood averages about 15.8 g./100 ml. in the male and 13.8 g./100 ml. in the female. The hemoglobin content of the blood decreases after blood losses from wounds or from impairment of the synthetic sites by chemical poisoning, or by abnormal destruction of red cells, as in spleen and liver misfunction. Anemias also develop as a result of diets deficient in vitamins, iron, or copper.

When the protein globin is removed, the iron-containing heme remains. Heme is an iron derivative of protoporphyrin, an intensely colored compound containing the porphyrin ring system (page 470). The iron in reduced hemoglobin is in the ferrous state. In oxygenated hemoglobin it is still in the ferrous state; the oxygen is assumed to be loosely linked to the iron by a residual valence force. Four molecules of heme unite with 1 globin to form hemoglobin as shown below.



The linkages involve the ferrous ions of heme and histidyl components of the globin moiety. Experiments have shown that each molecule of hemoglobin is capable of transporting 4 molecules of oxygen.

$$(\text{heme})_4\text{globin} + 4O_2 \rightleftharpoons (\text{heme } O_2)_4\text{globin}$$

The iron in hemoglobin can be oxidized to the ferric state, yielding the brown-colored methemoglobin. Methemoglobin, which may arise *in vivo* as a result of drug or poison action, is incapable of oxygen transport. Carbon monoxide combines with hemoglobin to form carbon monoxide hemoglobin, a complex with a much greater stability than oxyhemoglobin. If a significant portion of the hemoglobin is complexed in this manner, symptoms of anoxia develop, followed by coma and death. The metabolic elimination of the porphyrins is discussed in Chapter 20.

A hemoglobin, comparable in heme structure but containing a different globin constituent, has been reported in the root nodules of bacteria. Presumably this hemoglobin participates in nitrogen fixation (page 226) although the exact role has not been defined.

Leucocytes. White blood cells (leucocytes) are normal constituents of blood. In human blood the normal white cell count may fall as low as 5,000/cu. mm., and maximal normal counts of 10,000 are not uncommon. The average white cell count is about 7,000/cu. mm. of blood.

Under the microscope these cells are present in many characteristic forms. One type consists of small and large lymphocytes, which reach the blood via the lymphatic system. Others are described as large mononuclear, transitional, and polymorphonuclear, depending on the shape and appearance of the cell nuclei. Others are classified according to their staining characteristics. For example, eosinophiles are stained red with eosin, whereas basophiles are stained a blue color with methylene blue.

The polymorphonuclear leucocytes constitute about 65 to 70 per cent of the white cells of the blood. They possess amoeboid movement and have the ability to engulf and destroy bacteria and foreign substances in blood. This process is known as phagocytosis, and the amoeboid white cells are known as phagocytes. Consequently leucocytes function to combat infections. In acute infections the white cell count tends to increase markedly. In acute appendicitis, for example, the white cell count may be as high as 15,000 to 20,000/cu mm. of blood. A white cell count is used routinely to assist the physician and the surgeon in diagnosing this disease and other types of infections.

Blood platelets. The blood platelets are much smaller than either the leucocytes or erythrocytes. They vary in size from 1.5 to 3 μ. Their chemical composition is lipoprotein and simple protein in nature. Platelets are extremely unstable and difficult to isolate as they disintegrate on removal from the circulatory system. Platelets are thought to contribute thromboplastin, the substance essential for the conversion of prothrombin to thrombin in the initiation of the blood-clotting process.

Plasma and serum. The fluid portion of uncoagulated blood is called plasma. It can be isolated from whole blood by removing the formed bodies by centrifugation. Plasma contains a group of proteins including the albumins, globulins, and fibrinogen. If blood is allowed to clot, the fluid portion remaining is termed serum. Serum and plasma are similar in composition except that the fibrinogen is removed in the clotting process. Blood plasma contains about 90 per cent water, 6 to 8 per cent proteins, 0.85 per cent inorganic salts, and small amounts of glucose, triglycerides, phosphatides, cholesterol, and the nitrogenous constituents urea, uric acid, ammonium salts, creatine, creatinine, and purine bases. Table 13–1 lists the inorganic constituents of human plasma. The amounts of organic and inorganic constituents are subject to narrow fluctuations, depending on the state of nutrition and the activity of the individual. The non-protein nitrogenous components are largely waste products from cellular metabolism in transit to the kidneys and other organs of excretion.

TABLE 13–1. Inorganic Constituents of Human Plasma

Constituent	Per Cent
Chlorides as Cl	0.35
Sulfates as H_2SO_4	0.013
Phosphates as H_3PO_4	0.015
Potassium	0.031
Sodium	0.34
Calcium	0.01
Magnesium	0.007
Iodine, cobalt, etc.	Traces

Serum albumins and globulins are very similar in physical properties to the albumins and globulins obtained from eggs. Although the albumins and globulins isolated from different species have similar molecular weights and other physical properties, antigenically they appear to be distinct for each species. Serum globulins can be separated into a number of fractions, of which α-, β-, and γ-globulin are the most important. The γ-globulin fraction has received very extensive study as it contains a large portion of the antibodies (page 114) found in blood.

Serum proteins serve as buffers helping to regulate the pH of the blood. They are also important in maintaining proper water balance between blood and tissues. Serum albumin, because of its lower molecular weight, is especially effective in this respect. When food proteins are lacking, serum proteins appear to serve as a protein reserve from which essential amino acids can be drawn for use by other body tissues. Blood plasma, preserved by lyophilization, can be reconstituted by the addition of sterile water and used to replenish blood in the treatment of shock, burns, and injury. Prompt administration of plasma saved many lives during World War II.

Blood clotting. Although the fibrinogen content of blood is much smaller than that of the other plasma proteins (0.2 to 0.4 per cent compared with 4 to 5 per cent albumin and 2 to 3 per cent globulin), it plays a most important role in the organism. When a blood vessel is damaged, or when blood is drawn from a vessel, clotting or coagulation occurs within a very short time. Fundamentally, the formation of this clot is caused by the creation of stringy, insoluble fibrin from the soluble fibrinogen of the plasma. The stringy, jelly-like fibrin enmeshes the corpuscles, forming the clot and preventing excessive hemorrhage from small wounds. The importance of this process to the well-being of the animal is demonstrated by its absence in so-called bleeders, individuals who lack the factors necessary for normal blood-clot formation. This hereditary condition is called hemophilia. Clot formation is the final step in a complicated series of reactions which is not completely defined. In normal *in vivo* blood, fibrinogen remains in solution unless contamination with juices from damaged cells occurs. Coagulation may also be prevented by precipitation of calcium ions as the insoluble calcium oxalate.

Production of fibrin is the immediate cause of clotting. The soluble fibrinogen is hydrolyzed into smaller, insoluble fibrin protein and several small peptides, a reaction catalyzed by thrombin, a proteolytic enzyme. The enzyme thrombin is not present in normal blood, rather, it exists as prothrombin, an inactive precursor which can be converted into the active form through the action of thromboplastin. This reaction is also thought to be a proteolytic hydrolysis, yielding the thrombin plus a small peptide. Thromboplastin is apparently released from disintegrating platelets or other sources such as damaged brain tissue.

Blood contains an accelerator for the clotting process, known as accelerator globin or AC-globin. If one sets up a model system containing prothrombin, calcium, thromboplastin, and fibrinogen, the velocity of clotting is slow, but addition of a small amount of serum globulin accelerates the process by stimulating the conversion of prothrombin to thrombin. Blood also contains an inhibitor, heparin, which prevents the prothrombin conversion in circulating blood. Heparin is a low-molecular-weight carbohydrate containing glucosamine, glucuronic acid, and sulfate groups. Its structure has not been determined completely.

The clotting process may be summarized as follows:

$$1.\ \text{Prothrombin} \xrightarrow[\substack{\text{Ca}^{++} \\ \text{platelet accelerator} \\ \text{(inhibited by heparin)}}]{\text{thromboplastin}} \text{thrombin}$$

$$2.\ \text{Fibrinogen} \xrightarrow{\text{thrombin}} \text{fibrin} + \text{peptide}$$

$$3.\ n(\text{Fibrin}) \xrightarrow{\qquad\qquad} (\text{fibrin})_n \text{ polymer}$$

$$4.\ (\text{Fibrin})_n \text{ polymer} \xrightarrow{\qquad\qquad} \text{fibrin gel}$$

The peptide is removed on hydrolysis by thrombin and serves as a protecting or masking unit for the polymerization centers on the initial fibrinogen. Thrombin also functions in the conversion of a plasma globulin to a specific serum globulin. This conversion, as well as (1) and (2) above, is proteolytic in nature, whereas the third and fourth steps involve film formation through polymerization and the entrapment of the corpuscles in the fibrin web or mesh.

Thrombosis or intravascular clotting sometimes occurs and plugs small arteries or capillaries. Such clots in many regions of the body result in nothing more than a temporarily painful area. However, if they occur in the heart or the brain, death may result unless the clot is removed naturally or by the use of hemorrhagic drugs, heparin or hirudin (the anticoagulant in the saliva of leeches and ticks). In such cases it is often advisable to regulate the prothrombin synthesis (stimulated by vitamin K, page 359) through the use of antivitamin K, dicoumarol (page 361), or a derivative. The rodentocide, warfarin, is a derivative of dicoumarol and functions through the production of uncontrolled internal bleeding when consumed by rodents.

Gas exchange. The above discussion reported the oxygen-transport function of the hemoglobin of erythrocytes. Oxygen is absorbed by the blood as it passes through the fine capillaries in the thin wall of the lung. These capillaries are so small that the corpuscles must pass through single file and thus provide ample opportunity for gas exchange by simple diffusion. There is no evidence for "active transport" across the membrane. As was stated earlier, the absorbed oxygen forms a loose complex with the hemoglobin molecule. Experiments have demonstrated that the partial pressure of the oxygen in the small chambers of the lung (alveoli) is sufficient to account for the complete saturation of hemoglobin as blood passes through the capillaries. At the same time carbon dioxide, which was transported from the cells to the lungs in the venous blood, largely as sodium bicarbonate, diffuses into the lungs. The dissociation of the bicarbonate ion is aided by the slight change in the amphoteric properties of oxyhemoglobin. Oxyhemoglobin is slightly more acid than hemoglobin, so that the bicarbonate ion dissociates into water and carbon dioxide:

$$Hb^{-}O_2 + H_3O^+ + HCO_3^- + Na^+ \rightarrow Hb^{-}O_2 + Na^+ + 2H_2O + CO_2$$

There is no net change in the pH of blood as a result of this exchange.

In tissues of the body the reverse process occurs. Oxyhemoglobin, in a region of low oxygen tension, dissociates and oxygen diffuses across the capillary membrane to the surrounding tissue. Carbon dioxide of the tissue diffuses into the blood and is transported as the bicarbonate ion.

Nitrogen and the inert gases of air are also absorbed to a small extent. The degree of absorption is limited by their solubility in plasma. There is no change in nitrogen tension on passage through the arterial and venous network, indicating no utilization, whereas oxygen tension drops from 100 to 38 ml. of mercury and carbon dioxide rises from 35 to 43 ml. of mercury.

Lymph

In addition to the blood circulatory network there is another system, less well defined in distribution, known as the lymphatic system. This network contains a creamy, colorless liquid called lymph, through which a constant exchange between the blood and the tissue cells is maintained. Chemically, it is very similar to blood plasma. The suggestion has been made that it probably arises as a result of seepage of blood plasma through the capillary walls. Experiments using I^{131}-labeled serum albumin do not support the above suggestion, so the exact origin of lymph tissue remains in doubt.

Lymph spaces of all tissues are connected to lymph channels which carry the fluid to ducts. Lymph from the body tissues enters the blood through the thoracic duct, and that from the head enters through a duct in the neck region.

As stated above, lymph functions in the transport of nutrients and oxygen from the capillaries to the neighboring cells, again through diffusion processes. In addition, lymph functions in fat absorption (see also page 438). Fat is absorbed from the intestinal villi, via the lacteals, and passes by way of the lymphatic system to the blood through the thoracic duct. Waste products not absorbed by the capillaries also enter the venous blood stream for disposal.

SUPPORTING TISSUES

Bones

Bones form the skeletal framework of the body and are characterized, chemically, by a high proportion of mineral matter. In the embryo, bones appear first as cartilaginous structures consisting of cells embedded in a homogeneous intercellular substance, largely protein. The protein present in the largest amount is called collagen. Other proteins, present in smaller amounts, are osseomucoid and osseoalbuminoid. When ossification takes place, the intercellular spaces fill with mineral salts of which a salt closely related to tricalcium phosphate predominates. Although authorities are not in complete agreement, evidence indicates that the mineral matter in bone consists of a complex salt, the formula for which may be written $3Ca_3(PO_4)_2 \cdot CaX_2$. The carbonate radical $CO_3^{=}$ usually occupies the X_2 position, but other radicals, such as $2F^-$, $SO_4^{=}$, or $O^{=}$, may also constitute this part of the molecule. Magnesium can replace calcium in bone, but the amount of magnesium in bone appears to be relatively constant. Fluorine is always present in normal bone in small amounts.

Bone marrow, the principal source of red blood cells, consists of organic material containing a protein, called ossein, and relatively large amounts of fatty materials.

The normal mature bone is composed of nearly 50 per cent water, 20 to 25 per cent fat, 15 to 17 per cent ash, 7.5 to 10 per cent protein, and 1 per cent citric acid. On a moisture-free, fat-free basis, 60 to 70 per cent of the bone is inorganic in nature with the following composition:

Component	Man	Cattle
$Ca_3(PO_4)_2$	83.89%	86.09%
$Mg_3(PO_4)_2$	1.04	1.02
Ca (combined with CO_2, Cl, F, citrate)	7.65	7.36
CO_2	5.73	6.20

Appreciable amounts of additional inorganic components may be found in bone under special circumstances, such as lead in cases where animals have died of lead poisoning. The composition of bone can be influenced by a number of factors. Lactating animals in high production maintain normal amounts of calcium and phosphorus in milk at the expense of their skeletons, even when the diet is supplemented with these minerals. Periods of lowered production are needed to allow skeletal recovery.

The ossification process is hampered in rickets (page 357), osteomalacia, and similar bone diseases. These conditions may be initiated by calcium and phosphorus starvation, by unbalanced Ca : P ratio, by absence of sunlight or vitamin D (page 355), or by pathological conditions.

Teeth

The teeth are bony structures consisting of mineral matter embedded in an organic matrix. The principal constituents of teeth are enamel, cement, and dentine. Cement represents the layer covering the root of the tooth. It is a hard material similar to bone, containing about 70 per cent of mineral matter and an organic matrix consisting largely of collagen. The surface of the tooth is composed of an extremely hard material called enamel, which contains from 96 to 98 per cent of mineral matter with a small amount of organic material consisting largely of keratin. The main body of the tooth, which lies underneath the enamel and next to the pulp, is composed of dentine. This structure contains about 77 per cent of mineral matter and an organic matrix containing collagen.

Enamel, although similar to bone in chemical composition, seems to contain more calcium and phosphorus and less magnesium and water. Also, enamel is characterized by the presence of a hydroxyl group, which does not appear to be present in bone. Enamel has been described as inert, once deposited. However, recent studies with tagged elements have shown that radioactive phosphorus injected into an animal soon appears in the tooth and that about 10 per cent of it is found in the enamel layer. Dentine and cement occupy an intermediate position between enamel and bone, so far as chemical composition is concerned. Fluorine appears to be an essential constituent which provides a defense against dental caries.

There are two schools of thought regarding the cause and prevention of dental caries. One group emphasizes the importance of proper diet, involving quantity and quality of calcium and phosphorus and other dietary essentials such as fluorine and vitamins A, C, and D. The other group believes that dental hygiene plays the major role, that acid-forming bacteria flourish on carbohydrate residues, and that tooth structures are impaired by the action of acid.

Epithelial and Connective Tissues

These tissues serve to bind and hold the softer body tissues together. They are characterized by great strength, toughness, and elasticity. Epithelial tissues serve as a covering and lining for the body; for example, these tissues line the respiratory tract and alimentary canal. The outer covering of the body also consists of epithelial tissue and includes such epidermal tissues as skin, nails, hoofs, horn, hair, and feathers. All these tissues are composed of a tough, insoluble albuminoid protein, called keratin. The keratins of skin are known as pseudokeratins, whereas those in hair and nails are called eukeratins.

The most important connective tissues are ligaments, tendons, and cartilages. Tendons and ligaments consist of two types of tissue, namely, white fibrous tissue and yellow elastic tissue. The predominating components of tendons and ligaments are the albuminoid proteins, collagen and elastin. Collagen is a tough, insoluble protein which differs from keratin in that it can be hydrolyzed by digestive enzymes. It contains less sulfur than keratin and forms gelatin when treated with hot water. Commercial gelatin is manufactured from collagen-containing animal tissues.

Tendons contain relatively large amounts of collagen, an albuminoid which contributes strength. Another albuminoid in tendons is tendomucoid. Cartilaginous tissues contain elastin, chondromucoid, and chondroalbuminoid. Chondromucoid is a glycoprotein which yields, on hydrolysis, glucuronic acid, galactosamine, acetic acid, and sulfuric acid. Chondroalbuminoid has physical properties similar to those of keratin and elastin, but it can be hydrolyzed by proteolytic digestive enzymes.

MUSCLE TISSUE

This important tissue constitutes about 45 per cent of the body weight in man. From 50 to 75 per cent of the total body metabolism occurs in this tissue. The highest metabolic rate accompanies strenuous physical exercise, whereas metabolic activity is lowest during complete rest.

Muscle tissues can be classified into three types, depending on their physiological characteristics. Although they differ to some extent in their chemical properties, they have many chemical characteristics in common. The three types of muscles are (1) voluntary (striated, skeletal), (2) involuntary (smooth, unstriated), and (3) cardiac.

Voluntary muscle acts under conscious control. It is cross-striated with horizontal bandings quite visible under the microscope. The dark and light areas arise from differences in light refraction in the banded areas due to structural differences. The striated muscle constitutes the major fraction of muscle tissue. It contains fibers made up of long, spindle-shaped, nucleated cells held together by connective tissue fibers and enclosed in a connective tissue sheath to form bundles called myofibrils. Involuntary muscles are smooth and non-striated. They are composed of short, spindle-shaped, nucleated cells. These cells are also held together in a compact group by connective tissue fibers. Smooth muscles make up the skin and the walls of the bladder, arteries, and veins. Cardiac muscle is made up of irregular, sometimes branched cells which are striated in a manner similar to the voluntary muscles. The cells are truncate in appearance with a protoplasmic connection between cells.

Blood vessels are distributed throughout the muscle tissue in a fine web of capillaries so that each muscle fiber comes into close contact with them. Lymph surrounds and bathes every muscle cell, aiding in the transfer of nutrients to and waste products from the muscle fiber. Nerve endings are also found in each cell, thus linking the control center with the site of action.

Muscle Proteins

As is true of most biological substances, muscle is composed largely of water, about 70 to 75 per cent. Protein accounts for about 20 to 25 per cent of the fresh weight, with lipides, glycogen, glucose, and products of intermediate metabolism accounting for the remaining weight (about 5 per cent). Muscle proteins can be separated into five general types, myogen, myosin, globulin X, stroma, and actin. Myosin is soluble in salt solutions at pH 6.3 and an ionic strength of 0.62. Actin can be removed by salt solutions of similar pH but lower ionic strengths. Globulin X is extracted with salt solutions and precipitates when the salts are removed by dialysis. No function is evident for this protein. Stroma, the protein that remains after extraction of the fractions mentioned, forms the membranes of the muscle cells. The red color of muscles is due to small amounts of myoglobin, an iron-porphyrin-histone complex with properties similar to those of hemoglobin (page 293).

Myosin and actin form the contractile portion of muscle. Actin, embedded in the myosin fibers, exists in two forms, fibrous actin and globular actin. The fibrous actin forms a complex with myosin, yielding the viscous actomyosin. Fibrous actin is thought to be formed by polymerization of the globular precursor through the influence of ATP (page 110).

$$\text{Globular actin} + n\text{ATP} \rightarrow \text{fibrous actin} + n\text{ADP} + n\text{HPO}_4^{=}$$

Actomyosin is thought by some to constitute the adenosine triphosphatase found in muscle tissue. Others feel that ATPase and myosin are closely related and difficult to separate but are not identical. This concept is supported by inhibitor, activator, and denaturation studies. Myosin is a large molecule, with a molecular weight in the neighborhood of 1,000,000, while actin is much smaller, about 70,000 g./mole. Actomyosin has a molecular weight in excess of 1,000,000.

Actomyosin is a viscous substance and is birefringent. Threads spun from this protein by extrusion into a dilute salt solution show contraction in the presence of ATP, potassium, and magnesium ions.

Myogen is a water-soluble albumin type of protein found in muscle, although it is not involved directly in muscle contraction. However, it does contain a number of enzymes involved in carbohydrate metabolism. The enzymes detected in myogen include isomerase, aldolase, triosephosphate dehydrogenase, and phosphorylase.

Muscle Action

Contraction of muscle is brought about by the shortening or sliding of actomyosin molecules in the myofibril by a mechanism that is not completely understood. X-ray data show that the molecule is denser in the contracted state than in the extended form. Measurements also show a change in electrical potential. Different theories have been developed to explain these observations. One such theory assumes a stretching and compression of the actomyosin helix during relaxation and contraction, respectively. Another theory proposes a sliding filament model, with the actomyosin molecules sliding by one another, thus producing relaxation or contraction of the myofibril, depending on the direction of motion. The first theory suggests that actomyosin, as do all proteins, contains polar groups associated with the constituent amino acids. The interattraction of these polar groups, along with hydrogen bonding, influences the rigidity of the helix. If an ATP molecule is introduced, the three associated negative charges cause a reorientation of the interpolar attractions with contraction resulting. The energy for the contraction is supplied by the hydrolysis of ATP. Muscular contraction could be likened to the extension or compression of a coil spring as shown in Figure 13–1. Other workers have suggested that a combination of positive and negative groups in the protein is involved in the contraction and that ATP functions to prevent the interaction within the molecule through the contribution of the three negatively charged groups.

The sliding filament theory is based on the observation that actin and myosin must be bridged or combined in order to form contractile threads. By selective treatment it is possible to remove the myosin and the actin portions separately, and by use of the electron microscope to determine the dimensions of each unit in the myofibril. Studies of electron micrographs show no evidence of significant decrease in length of myosin or actin during contraction. If actin and myosin slide by each other forming new points of attachment (Figure 13–2), muscle contraction or expansion can occur without assumption of helix expansion and contraction. ATP again is presumed to function in the formation of the oscillating bridge. All the theories advanced agree that hydrolysis of ATP into ADP plus inorganic phosphate provides the energy required for muscular contraction, but little progress has been made toward explaining the mechanism of the conversion of about 8,000 cal./mole of chemical energy into mechanical energy.

The ATP required for muscle contraction is derived from aerobic oxidation of glycogen or other food reserves. In some muscles, flight muscle of blowfly, for example, the myofibrils are separated by layers or rows of mitochondria for rapid oxidation of food reserves. Under anaerobic conditions the soluble glycolysis enzymes metabolize carbohydrates to the lactic acid stage. This acid accumulates until aerobic conditions return, when lactic acid is metabolized completely.

During muscle contraction there is a movement of potassium ion from the muscle cells to the extracellular fluid (Figure 13–1). On

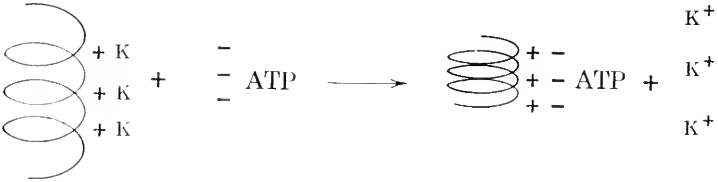


FIGURE 13-1. Schematic drawing of muscular contraction through helix or "coiled spring" compression. The nature of the forces binding the potassium ions to the extended muscle is unknown, but some reorientation of the polar groups on the molecule is necessary to release them.

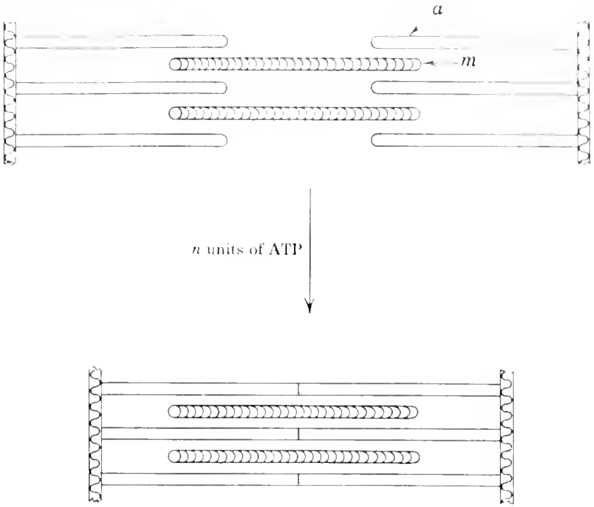


FIGURE 13-2. Schematic drawing of muscular contraction involving the sliding-filament theory of Huxley and coworkers; *a* in the drawing represents actin, *m* represents myosin. ATP molecules function by bridging between the actin and myosin at successive points as contraction occurs, with hydrolysis of the energy-rich phosphate compound supplying the energy required for contraction. In the absence of ATP the muscle components remain locked in position as in rigor mortis.

relaxation, this ion returns to the muscle tissue. Potassium is essential for normal muscular function although the mechanism of action is obscure.

NERVOUS TISSUE

Although nerve tissues make up only about one-fortieth of the total weight of human beings, this type of tissue is without doubt the most important in the body, for by its functions man and lower animals are clearly differentiated. Nerve tissue of the spinal cord, peripheral nerves, and ganglia provide the channels through which the brain exercises control over most of the functions and actions of the body either directly by nerve impulses or indirectly by nervous control of the blood supply.

Composition

Brain and other nervous tissues differ materially in certain respects from most other body tissues, but as usual water is the major component on a weight basis, with brain tissue containing as much as 80 per cent. Of the solids present, the lipide fraction is of interest since it contains particularly large quantities of the phospholipides lecithin, cephalin, and sphingomyelin. Glycolipides (cerebrosides), cholesterol, and sulfolipides comprise the remaining portions of the lipide fraction. Triglycerides do not occur in quantity in nerve tissues. The lipide fraction of the brain accounts for 30 to 40 per cent of the dry weight.

Proteins constitute about 50 per cent of the dry weight of nervous tissue, including small quantities of albumin, several globulins, and nucleoprotein. The major component is a keratin-like protein called neurokeratin, containing from 25 to 50 per cent lipides. The protein and lipide components form alternate concentric layers in the nerve fiber, so that a cross section of the nerve shows rings similar to those observed in a cross section of a tree trunk.

Nerve tissue also contains appreciable quantities of calcium, magnesium, and sodium and is exceptionally rich in potassium. When the nerve is stimulated, potassium diffuses into the surrounding fluid; during rest it diffuses back into the fiber.

Nervous tissue contains the usual extractives associated with metabolically active tissue. Creatine, lactic acid, purines, inositol, and other vitamins and cofactors have been isolated. However, only small quantities of glycogen are found in the brain. Therefore, nerve tissues must depend on a continual supply of blood glucose as a source of energy. In conditions where blood sugar drops, as, for example, during insulin hypoglycemia, patients become dizzy and confused, and in severe cases lapse into coma.

Nerve Action

Nerve impulses are transmitted from the site of origin to the region of action through a series of neurons. The neuron consists of the nerve cell, dendrites, and the axon (Figure 13–3). Dendrites are the small terminal branches of the nerve fiber which pick up the original impulse for transmission through the axon to the site of action. Motor neurons conduct impulses from the brain to areas of contraction and secretion. Sensory neurons receive the stimuli from the sense organs for transmission to the brain. Motor neurons and sensory neurons are interconnected by the interlacing of the dendrites with the fine branches of the sensory fibers. These connections are called synapses.

Although our knowledge of chemical reactions involved in nerve transmission is quite deficient, there is evidence showing the involvement of acetylcholine in the parasympathetic nerve system. This substance accumulates in nervous tissue during rest periods and

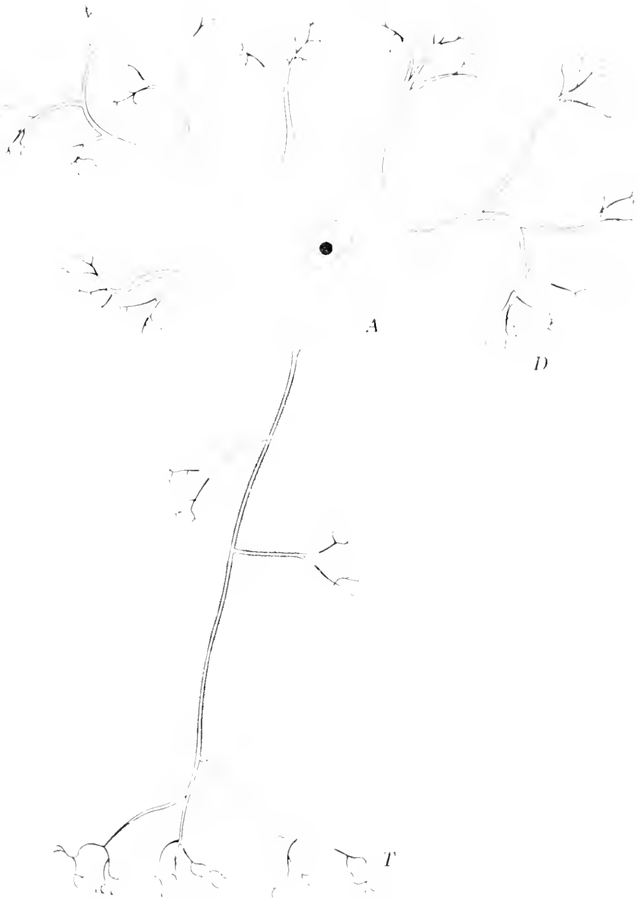
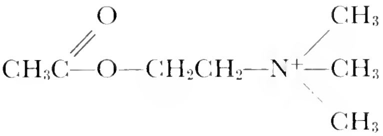


FIGURE 13-3. Motor neuron showing the nerve cell with dendrites (*D*) which pick up the impulse for transmission through the axon (*A*) and terminal arborizations (*T*) to the site of action. Such cells transmit impulses only in the direction of the terminal arborizations.



$$CH_3C(=O){-}O{-}CH_2CH_2{-}N^+(CH_3)_3$$

acetylcholine

decreases sharply on transmission of an impulse (Figure 13–4). Young rats contain 7 to 12 mμ moles of acetylcholine per g. of brain tissue. With the application of current across inserted electrodes, this level

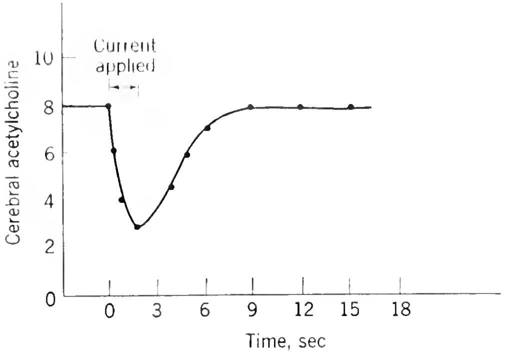


FIGURE 13-4. Brain acetylcholine in young rats before and after shock.

drops to 3 to 5 mμ moles. The loss occurs by a simple hydrolysis to choline and acetic acid and is brought about by choline esterases. Much of the acetylcholine in the brain is in a bound form and is protected from hydrolysis by choline esterase until release by initiation of the stimulus. In this respect the release of acetylcholine parallels that of potassium ions (see above). Acetylcholine is synthesized from acetyl coenzyme A and choline. The acetyl coenzyme A may arise from glucose metabolism or from a synthesis involving coenzyme A, ATP, and free acetate. The balance between synthesis and breakdown is shown in Figure 13–5.

The ATP required for recycling of acetate in the above synthesis is

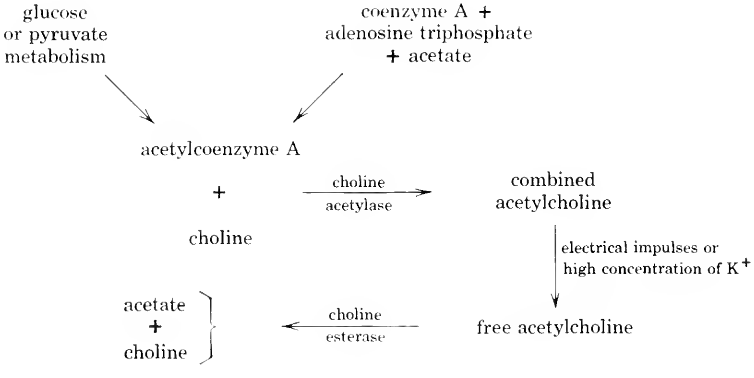
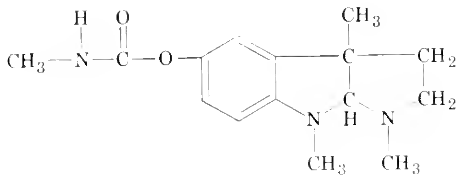


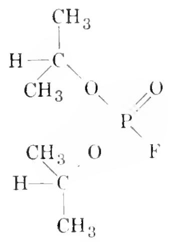
FIGURE 13-5. Acetylcholine breakdown and synthesis in brain tissue.

derived from oxidative phosphorylation or from transphosphorylation from phosphocreatine. These high-energy phosphate compounds increase slightly during periods of nerve inactivity, although the level remains fairly constant. Respiration increases during periods of stimulation to bring about recovery whenever quantities of ATP are needed for acetylcholine resynthesis.

The hormones noradrenaline and adrenaline (page 315) occur in the central nervous system and play a role in the sympathetic nervous system similar to that of acetylcholine in the parasympathetic system. An amine oxidase occurs in cerebral tissues and limits the action of adrenaline by oxidation to the corresponding aldehyde in a manner analogous to the hydrolysis of acetylcholine by choline esterase in the parasympathetic system.

The use of specific inhibitors has aided in the study of the effects of acetylcholine and adrenaline on nerve transmission. Eserine is a reversible inhibitor of choline esterase. Addition to an *in vitro* system results in alteration of the action potential; it may be removed from the nerve by washing, with restoration of conduction. Diisopropylfluorophosphate is an irreversible inhibitor of choline esterase and of nerve action. Many of the newer organic phosphate insecticides presumably function through this type of inhibition. Ephedrine and benzedrine are amine oxidase inhibitors and thus interfere with the normal enzymatic conversion of adrenaline and noradrenaline.


eserine (physostigmine)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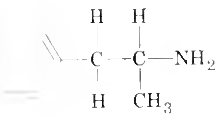
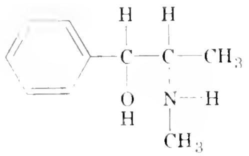

benzedrine


ephedrine

RESERVE TISSUES

The tissues discussed in earlier sections in this chapter constitute the working tissues of the body. Their functions are structure, transportation, and control. Certain other cells in the body function as storage areas for reserve foods to be used in time of stress. Small amounts of carbohydrates are stored as glycogen in the liver, muscle, and, to a much lesser extent, in the nervous tissue. The principal energy-storage form, however, is fats.

Fatty Tissues

Practically every tissue in the animal body contains lipides in some form, although certain tissues are characterized as fatty tissues. If an animal is starved for a long time, much of the lipide fraction is utilized, except for the phosphatides, which remain almost intact. Thus this latter fraction cannot be considered an energy reserve.

Reserve lipides are stored in areas characteristic of the animal in question. All animals store subcutaneous fats to a degree, and in cattle and hogs this is the major storage area. Man accumulates most of the adipose tissue in the abdominal region. Each species tends to store a characteristic mixture of fats, although the composition can be changed by feeding large quantities of a specific lipide, for example, linoleic acid-rich soybean oil. Pork fat contains triglycerides high in oleic acid with smaller amounts of stearic acid. However, if pigs are fed quantities of unextracted soybeans, they tend to accumulate greater amounts of linoleic acid, and the pork becomes flabby or oily. Other experiments have shown that similar changes can be produced in a number of species. When animals are fed ordinary rations, they tend to accumulate depot fats with melting points close to their own body temperature.

Subcutaneous fats usually contain more oleic acid than the visceral fats, which accumulate a higher percentage of saturated acids. It is obvious that an animal must oxidize unwanted fatty acids or alter them before storage in the particular depot in forms characteristic of the animal.

Although fatty tissues are quite inert from the metabolic standpoint, they are not entirely inactive. Experiments with fats containing deuterium (heavy hydrogen) in place of normal hydrogen have shown that there is a turnover of storage fats. Even small amounts of tagged fatty acids in the diet are first incorporated into the body fat before they are oxidized.

Although depot fats are stored primarily as food reserves, there are secondary benefits. Subcutaneous fats serve as insulators for the body. Lipides serve as shock absorbers in protecting joints, nerves, and other organs against mechanical injury.

GLANDULAR TISSUES

Certain tissues in the body are capable of elaborating and discharging secretions of the utmost importance to the welfare of the animal body. These are known as glandular organs, and they possess specialized cells known as gland cells. The glandular tissues do not, of themselves, differ strikingly in chemical composition from other body tissues, but they are of interest because of the chemical substances they elaborate.

These tissues may be classified into two groups: (1) glands of external secretion, with ducts which secrete their chemical materials in such a manner that they may pass from the body, and (2) glands that possess no ducts and secrete their chemical materials directly into the blood stream, whence they are carried to other tissues. The latter are known as ductless glands, endocrine glands, or glands of internal secretion.

Glands of External Secretion

This group of glands is of great importance to the body since the secretions are used for the digestion of food, the feeding of the young, or the excretion of waste products. External excretory glands related to assimilation are discussed in some detail in other portions of this book. They include the salivary, gastric, intestinal, pancreatic, and liver glands and are discussed in relation to their roles in providing enzymes and activators required for digestion (Chapter 17).

Sweat glands are located in the external epithelial tissue (skin) and serve as excretory organs, although their role is less significant than that of the kidney. They are tubular coiled glands whose ducts are controlled by smooth muscles which also aid in expulsion of sweat. They are most abundant in the palms of the hands and soles of the feet with lesser numbers in other surface areas of human beings. The foot pads of dogs and cats contain the only sweat glands found in those species.

Sweat contains about 99 per cent water. The main solid constituent is sodium chloride, although small amounts of other inorganic ions such as phosphates and sulfates are present. Urea is present in very low concentrations along with traces of lactate, uric acid, creatine, and volatile organic sulfur compounds.

The skin also contains sebaceous glands at the roots of the hair. These glands provide an oily secretion which prevents brittleness of hair.

Glands of Internal Secretion

Ductless glands, also called endocrine glands or endocrine organs, are unique in that they manufacture and secrete into the blood stream chemical substances necessary for the stimulation and regulation of other organs or metabolic processes. The chemical constituents released are called hormones. Their roles in metabolism are similar to those of the vitamins (Chapter 14) with the only real differentiation based on the fact that vitamins must be obtained from external sources whereas hormones are produced *in situ*. Hormones, as is true of vitamins, vary extremely in complexity from rather simple compounds like adrenaline and thyroxine to sterols and proteins. Many of the biologically active endocrine secretions remain to be chemically identified.

Gastrointestinal hormones. The hormones produced in the digestive tract play an active role in mobilizing the forces required for digestion of foods (Chapter 17). Gastrin, produced by the pyloric mucosa in response to stimulation by certain nitrogenous constituents of food entering the stomach, stimulates the active hydrochloric acid secretion from the fundic cells. Histamine functions in the stimulation of the flow of gastric juices. But there is good evidence that gastrin and histamine are synonymous.

When hydrochloric acid of the acid chyme reaches the small intestine, cells of the intestinal mucosa are stimulated to produce the hormone secretin. This hormone is carried by the blood to the pancreas, where it stimulates the flow of pancreatic juice containing sodium bicarbonate to neutralize the chyme. Secretin has been obtained in crystalline form. It is a polypeptide of unknown structure.

Simultaneously a second hormone, pancreozymin, stimulates the production of the pancreatic enzymes (page 434). Pancreozymin is also polypeptide in nature. It can be separated from an alcohol solution also containing secretin by saturation with sodium chloride. A third hormone, enterogastrone, also originating in the intestinal mucosa, is secreted in response to the presence of fatty foods in the intestine. It functions by slowing down the discharge of the gastric chyme so as to permit more complete digestion of the lipides in the intestine.

Extracts of intestinal mucosa contain a factor which stimulates the contraction of the gall bladder. This hormone, cholcystokinin, appears to resemble secretin chemically in that it is a basic polypeptide with similar solubility and electrophoretic properties.

Pancreas. This organ functions not only as a source of pancreatic juice but also as an endocrine tissue. When this gland is removed, animals develop diabetes, a metabolic disorder in which the body loses its power to oxidize sugars and fats. In this disease the blood sugar becomes abnormally high (hyperglycemia) and glucose is discharged into the urine. The latter condition is called glycosuria. The pancreatic hormone, which prevents diabetes, has been isolated in crystalline form and is known as insulin.

Insulin is secreted by special areas of cells in the pancreas, known as the islands of Langerhans. When these cells become diseased and fail to function, insulin is not secreted and diabetes results.

The complete amino acid sequence in the insulin molecule has been elucidated in a classical study by a group of English biochemists. It is a protein with a molecular weight of 12,000 g./mole. The protein can be split by mild reduction into two pairs of different peptide chains. The four peptide chains are cross linked through disulfide bonds (—S—S—). If these linkages are destroyed by reducing agents, such as hydrogen sulfide or cysteine, resynthesis of an active hormone by oxidation does not occur. This has led to the suggestion that folding occurs in the original chains, and that on release the chains shift so that proper orientation for the cross links of disulfide groups is not possible.

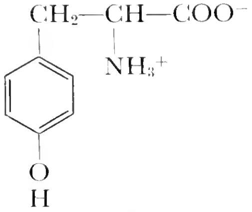
Insulin used for medical purposes is prepared commercially from pancreatic glands of sheep, beef, and hogs. It cannot be administered orally because it is destroyed by proteolytic enzymes. Therefore it is injected in the form of the water-soluble hydrochloride. Such insulin disappears rather rapidly so that control of blood sugar is only temporary. However, the disappearance can be delayed by injecting a zinc-protamine-insulin complex or a globin-zinc-insulin complex. These forms are slow to act. Frequently a mixture of the regular and slow-acting types is injected to get both immediate and prolonged control. If injection of excessive quantities lowers the blood sugar to the point where insulin shock occurs, injection or oral consumption of glucose is used to overcome the excess. Insulin shock therapy has been reported beneficial in the treatment of schizophrenia, but the results are still in doubt.

Insulin functions in stimulating the normal oxidation of sugar and the normal deposition of glycogen in liver and muscle. It is not essential in the known schemes of glycolysis since glycogen deposition can occur in the muscles of pancreatectomized dogs. Also, the synthesis of glycogen *in vitro* by isolated enzyme systems does not require, or respond to, insulin. An earlier, attractive suggestion that insulin is directly involved in the glucokinase system is not supported by experimental evidence, although an indirect role through counteraction of the inhibitory influence of the adrenohypophysis and the adrenal cortex has not been eliminated. The suggestion has also been made that insulin is necessary for oxidative phosphorylation. Here again, direct involvement has not been demonstrated in *in vitro* systems. Insulin has been implicated in glucose transport across cell membranes. Glycogen formation by rat hemidiaphragms is stimulated by insulin addition. At the same time, lactic acid production is not affected. This observation has led to the suggestion that the glycolytic pathway stimulated by insulin is a route separate and distinct from the classical Embden-Meyerhof pathway.

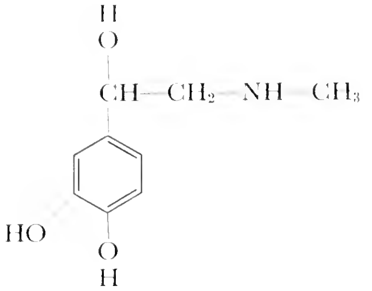
Until more knowledge is available it will be impossible to define the function of insulin in metabolism. The role of this hormone may well be multiple in nature.

Adrenal glands. The adrenal glands, sometimes called the suprarenal glands, are small bodies, one situated on the upper end of each kidney. In human beings each gland weighs about 3 g. The adrenal consists of an inner portion, the medulla, an offshoot of the sympathetic nervous system, and an outer portion, the cortex. Each anatomical portion has its own endocrine characteristics.

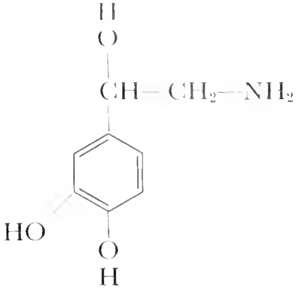
ADRENAL MEDULLA. Two hormones are secreted by the medulla: adrenaline, or epinephrine, and noradrenaline, or norepinephrine. The chemical structures of these two hormones are closely related to that of the amino acid, tyrosine.



tyrosine



adrenaline



noradrenaline

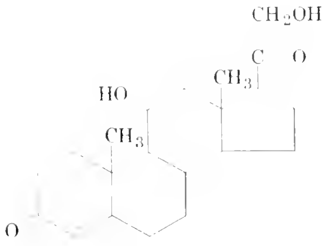
Phenylalanine has been shown to be a precursor of adrenaline and noradrenaline. Synthesis involves oxidation of the aromatic ring and side chain and decarboxylation. The conversion of noradrenaline to adrenaline by a methylation reaction involving ATP has also been suggested.

When adrenaline is injected it causes constriction of the arterioles, increases blood pressure, and stimulates the heart. In anger or fear the amount of adrenaline automatically increases, followed by a marked increase in blood sugar and lactic acid. The natural L form is 15 times more effective than the D form. A solution consisting of 1 part of adrenaline hydrochloride to 300,000,000 parts of water causes a physiological response in animals.

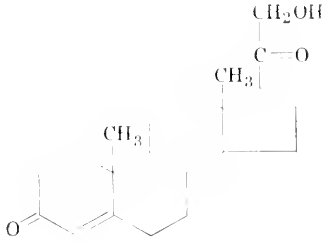
Noradrenaline and adrenaline are presumed to function in the transmission of nerve impulses in the sympathetic nervous system (page 309). However, alternate systems must be available, as the medulla can be removed without harm.

ADRENAL CORTEX. Although the medulla does not appear to be essential for life, animals perish if the adrenal glands are removed because the cortex plays an important physiological role. Loss of the cortex by surgical removal or by disease results in loss of sodium, increased blood potassium, decreased glycogen in liver and muscle after fasting, decreased glucose in serum, and inability to excrete ingested water. Emaciation, low blood pressure, and abnormal pigmentation of the skin result. These findings are similar to those observed in Addison's disease in man. Hormones in cortical extracts prevent and cure this disease.

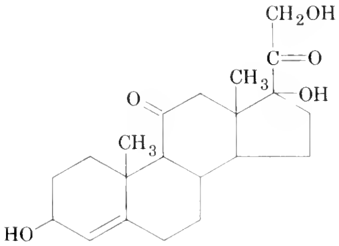
About 30 steroids have been isolated from the adrenal cortex. The chemical configurations of these have been determined, and several of them have been synthesized. The hormones may be grouped into two classes from the functional standpoint: (1) those with predominant action on electrolyte and water regulation, and (2) the corticoids, which exert their influence on carbohydrate metabolism. Deoxycorticosterone is an example of the first class, and corticosterone of the second.



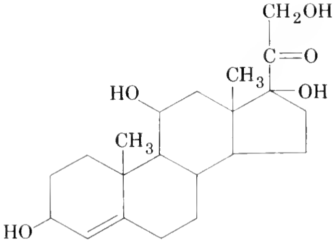
corticosterone



deoxycorticosterone



17-hydroxy-11-dehydrocorticosterone
(compound E)



17-hydroxycorticosterone
(compound F)

Compound E has been used extensively in the treatment of arthritis and rheumatism. It was first synthesized by a group of workers at Mayo Clinic and is produced commercially by a number of companies. Compounds E and F also have an effect on carbohydrate metabolism and stimulate glycogen deposition in animals previously starved. A number of synthetic steroid hormones, modified slightly by substitution of other groups such as halogens, are effective against arthritis and other diseases without some of the adverse side effects which result from extended use of compound E. The adrenocorticosterones are structurally quite similar to some of the sex hormones and might be interconverted *in vivo,* or administration of these hormones may stimulate the production of adrenogenital hormones which occur normally in small amounts. Whatever the reason, increased femaleness in the male (enlarged breasts with genital atrophy) and development of maleness in the female have been observed in some patients on adrenal hormone therapy.

None of the individual steroids cures Addison's disease, although the disease responds to corticular extracts, indicating that unidentified factors remain to be detected. It is interesting to note the great differences in biological activity observed upon minor changes in the steroid molecule. Substitution of an hydroxyl (—OH) group for hydrogen in position 11 changes the molecule from a mineralocorticoid to a glycocorticoid. Furthermore, only minor additional changes lead to a variety of sex hormones.

Thyroid gland. This gland consists of two lobes connected by an isthmus, and in man is attached to the trachea on both sides of the larynx. Before the use of iodized table salt became common practice, enlargement of this gland occurred in iodine-deficient areas, with the production of a large lump on the neck. This malady, called goiter or "big neck," was common in the north-central areas of the United States, which are not replenished by iodine carried inland by sea spray. Normal human thyroids (25 to 30 g. total weight) contain from 12 to 25 mg. of iodine, whereas glands of goiterous patients show little or no iodine. The amount found in normal thyroids varies with the age, the season of the year, and the iodine content of the food. Goiter is not peculiar to man but occurs also in other animals and has been produced in fish.

If the thyroid gland does not develop in early life, children become dwarfed and are known as cretins. The condition itself is known as cretinism. These abnormal dwarfs have low mentality, bowed legs, coarse hair, and thick skin. Hypothyroidism in adults leads to a condition known as myxedema. Symptoms include puffy swelling or edema, general depression of all bodily functions, and usually a depressed mental condition. These cases usually respond dramatically to the administration of desiccated thyroid glands or thyroid hormone. Hypothyroid cases exhibit abnormally low metabolic rates.

An abnormally active thyroid gland causes a disease known as exophthalmic or toxic goiter. Hyperthyroidism produces bulging eyes, nervousness, irritability, and insomnia. The metabolic rate is higher than normal, so patients burn their food reserves more rapidly, lose weight, and may succumb to cardiac failure unless a portion of the overactive gland is removed.

The chemistry of thyroid hormones is discussed on page 379. The mechanism by which thyroxine or other active derivatives function in controlling metabolism remains unknown. The action of the hormone has not been related to specific biochemical reactions in metabolism. Erythrocyte synthesis is stimulated by consumption of thyroxine, as is the case of lipide metabolism. Development changes such as metamorphosis of tadpoles are affected by extracts of thyroid tissues. Injections at early stages lead to the development of tiny frogs no larger than flies.

Parathyroid glands. These glands are so closely associated with the thyroid gland that very skillful surgery is required to separate them. In human beings there are at least four types of parathyroid tissues or glands. Two of these, the internal parathyroids, are embedded in the thyroid glands, and two others, the external parathyroids, lie behind the thyroid gland.

When the parathyroids are removed, experimental animals die within 2 weeks with many typical symptoms, the most distinctive of which is an increase in blood phosphorus and a lowering of blood calcium, accompanied by characteristic muscle contractions (tetany).

The administration of parathyroid hormone (parathormone) relieves tetany, increases blood calcium, decreases blood phosphorus, and increases the phosphatase activity of blood serum. Occasionally a tumor develops on the parathyroid glands, causing hyperparathyroid activity. This is known as von Recklinghausen's disease. In this disease, bones become decalcified and blood calcium values are abnormally high.

The parathyroid hormone is protein in nature and has a rather large molecular weight of 500,000 to 1,000,000, although an active fraction with a weight of 20,000 g./mole has been isolated.

The biochemical mechanism involved in calcium-phosphorus control has not been elucidated.

Pituitary gland. This small gland, also called the hypophysis, is situated at the base of the brain. Anatomically it consists of three structures, the anterior lobe, the intermediate lobe or pars intermedia, and the posterior lobe. The anterior lobe is the largest and most important portion of the gland, although the posterior lobe is also significant from the standpoint of endocrine activity. Not much is known regarding the hormone-producing characteristics of the pars intermedia. One hormone, intermedin, has been isolated which, when injected into a minnow, causes a red color at the point of attachment of the thoracic, abdominal, and anal fins. No function has been suggested for this hormone in higher animals.

So many important physiological functions have been attributed to the pituitary gland that it is difficult to formulate a satisfactory summary of its more important functions. If the pituitary is removed, a series of abnormalities becomes evident. Young mammals cease to grow, and physical and mental activities are retarded. If the gland is removed from the mature mammal, other endocrine glands tend to atrophy and diminish in hormone production. This is true for the thyroid, the parathyroids, the adrenals, the testes, and the ovaries. Appetite decreases, followed by loss of weight, and protein, fat, and carbohydrate metabolism becomes abnormal. In other words, this "master gland" seems to control the activity of most of the other endocrine organs.

ANTERIOR LOBE. Reduced activity in the anterior lobe of the pituitary gland leads to dwarfism in young animals, accompanied by retention of infantile characteristics and lack of sexual development. Administration of extracts of the anterior lobe prevents or alleviates these symptoms. Hyperactivity of a growth hormone in the anterior lobe causes gigantism, a condition in which young mammals tend to grow to gigantic proportions. Such individuals may be quite normal mentally and physically, although in some cases bone malformations may occur. This condition is evinced by overgrowth in bones of the face, hands, and feet of human beings (acromegaly), in addition to excessive growth of fibrous tissue, resulting in thickened nose, eyelids, and fingertips.

A protein has been isolated from ox pituitary glands which causes growth in hypophysectomized rats. It is without influence on depancreatized animals unless administered along with insulin. Since insulin alone has no such effects, some workers believe that the growth hormone stimulates the synthesis of insulin and that the two hormones combine to exert an inhibiting effect on carbohydrate and protein catabolism, thus making these substances available for growth.

The anterior lobe possesses a hormone which stimulates growth of the adrenals. The adrenocorticotropic hormone (ACTH), a protein of 20,000 molecular weight, has been isolated from the anterior lobes of a number of animals. It has been used medicinally for the treatment of arthritis and rheumatism. Presumably it functions through the increased synthesis of hormones such as compound E (page 316). The normal production of ACTH may be under the control of noradrenaline (page 315) and adrenaline. In times of stress the level of the latter hormones increases in the blood, leading to increased synthesis or release of the pituitary hormone.

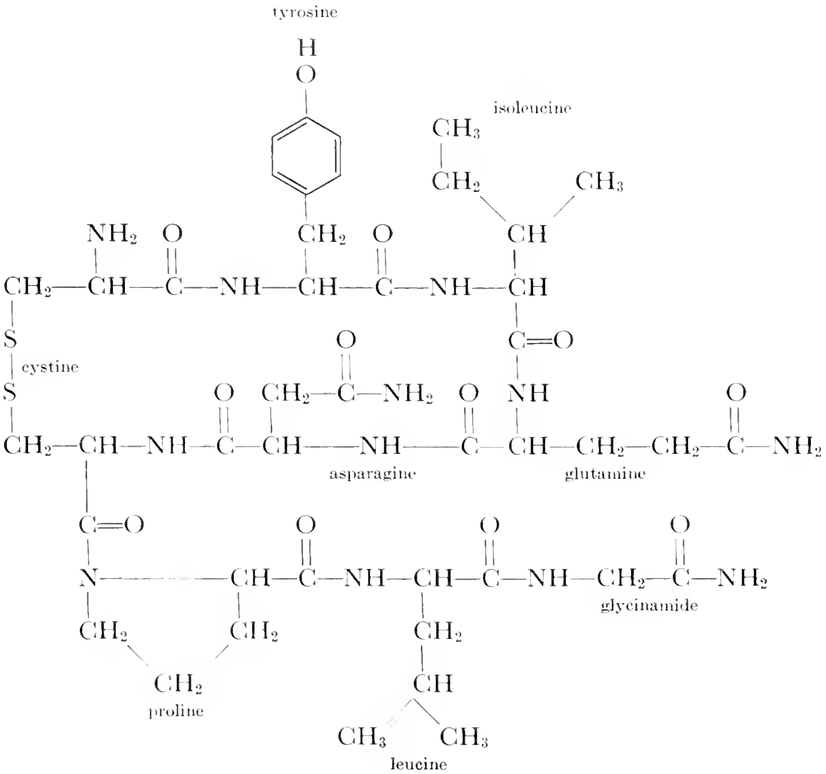
A thyrotropic hormone which stimulates the release of thyroid hormone has been identified. It is a small protein (10,000 g./mole) which on injection into an animal produces an enlargement of the thyroid gland, a rise in basal metabolism, and other symptoms of an excess of thyroid hormone.

Other hormones isolated from the anterior lobe of the pituitary include (1) the lactogenic hormone, prolactin, a protein which stimulates lactation in mammary glands suitably prepared by gonadal hormones, and (2) several gonadotropic hormones which stimulate follicular development in preparation for ovulation and control the development of the corpus luteum.

The hormones thus far isolated from the anterior lobe of the pituitary have all been proteins of medium molecular weight. All of them exert a control effect on other glands with secondary effects on metabolic reactions. Further research will undoubtedly show the existence of additional control factors in this portion of the pituitary gland.

POSTERIOR LOBE. Information on the control functions of the posterior lobe is meager and is hard to obtain because of the difficulty in removing that portion of the pituitary without seriously injuring the anterior lobe. Present evidence on apparently successful operations indicates a controlling action on urinary output and water intake and on smooth muscles in general. On removal of this lobe both water excretion and water intake increase in a manner similar to that encountered in *diabetes insipidus*. Many cases show improvement on administration of posterior lobe extracts.

Much of the information available on the action of the pituitary gland has resulted from observations of the pharmacological effects of injection of extracts or fractions thereof upon normal animals. Such



oxytocin

experiments provided the impetus for the isolation of the peptides, oxytocin (pitocin) and vasopressin (pitressin). These peptides were first separated in electrophoretic studies.

Oxytocin contains a ring of five amino acids linked to a side chain of three amino acids with all carboxyl groups in amide forms. The structure has been established not only by sequential amino acid determinations but also by synthesis. Oxytocin stimulates the contraction of the uterus and is useful in instigating the removal of the by-products of parturition. Oxytocin also functions in milk ejection from lactating mammary glands by stimulating contractions of the smooth muscles of those organs.

Vasopressin is also peptide in nature, containing eight amino acids, and is very similar to oxytocin except that phenylalanine replaces isoleucine in the cyclic portion of the molecule and arginine replaces leucine in the side chain in beef vasopressin. Lysine replaces leucine in the side chain of hog vasopressin.

Vasopressin causes arterial constriction (pressor action) with a rise in blood pressure. Administration of this hormone has been used to combat low blood pressure in treating shock. Vasopressin may also be functional in the constriction of renal tubes and thus prevent excessive water excretion, although some workers believe that a third peptide in pituitary extract is more effective for this purpose.

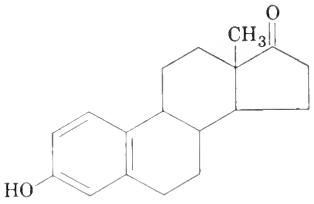
There is some evidence that the above hormones exist in the pituitary as one unit, and that the active principles isolated are the result of breakdown of that constituent. A protein, apparently pure, has been isolated which possesses activities associated with the above two (or three) peptides, that is, pressor action, uterine contraction, and renal action.

Placenta. Placental tissue contains a gonadotropic hormone, known as chorionic gonadotropin. This hormone is produced by the placenta and is excreted into the urine soon after pregnancy occurs. When pregnancy urine is injected into immature female mice, it causes hemorrhage of an ovarian follicle within a period of 4 days. This effect has become the basis of the Ascheim-Zondek pregnancy test, which has achieved real success in human medicine. The Friedman pregnancy test is based on the same principle. Human pregnancy urine is injected into mature unmated female rabbits. If the placental hormone is present in the urine, follicular rupture and corpus luteum formation occur within 1 or 2 days. The hormone is a glycoprotein containing galactose.

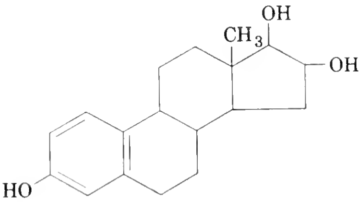
Reproductive glands. The organs of reproduction responsible for the production of sex hormones are the ovaries and the testes.

OVARIES. The cyclic phenomenon of menstruation in the human female is stimulated by a hormone called estrone. This hormone is produced in the growing ovarian follicle. The growth of the follicle, in turn, is stimulated by a gonadotropic hormone produced by the anterior lobe of the pituitary gland. The rupture of the follicle liberates the ovum, which passes to the uterus by way of the Fallopian tube. The ruptured follicle then develops into a yellow body called the corpus luteum, which in turn produces a hormone called progesterone. If the ovum is not fertilized, the corpus luteum grows for a few days and then gradually disappears to be replaced at monthly intervals by new follicles and ova.

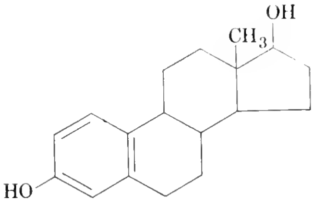
Modern chemistry has identified a number of estrogenic compounds, among which are (1) estrone or theelin, (2) estriol or theelol, (3) estradiol or dihydrotheelin, (4) equilin, and (5) equilenin. These estrogens are steroids.



estrone

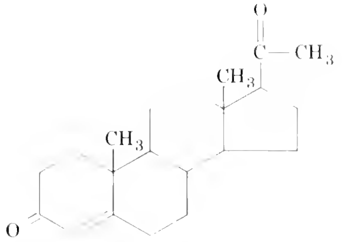


estriol



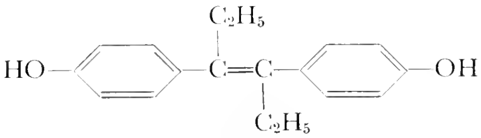
estradiol

Some investigators believe that the more potent estradiol is the true ovarian hormone which is converted to estrone, and that estriol is formed from estrone. The body seems to use glucuronic acid to detoxicate estriol, forming physiologically inactive estriol glucuronate, which is excreted. The corpus luteum hormone, progesterone, continues uterine changes which were initiated by the estrogenic hormones, estrone and estradiol. Implantation of the fetus on the wall of the uterus does not occur if progesterone production is lacking.



progesterone

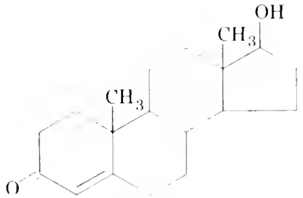
Stilbestrol is a synthetic estrogen derived from stilbene ($C_6H_5 \cdot CH : CH \cdot C_6H_5$). It is not chemically related to the natural estrogens, but the diethyl derivative is about three times as potent as estrone. This compound has found wide use in modern medical practice as a supplement to the natural supply of estrogens.



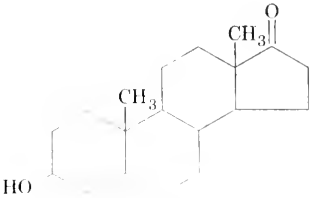
diethylstilbestrol

Many plants contain estrogenic substances in varying quantities which may be factors in the growth of animals. The administration of sex hormones to ruminants, swine, and poultry increases the growth rate and efficiency significantly. Hormones are implanted subcutaneously for slow release or given by mixing with the feed (page 421). Diethylstilbestrol is used quite extensively in animal and poultry husbandry. This hormone presumably induces the tranquilization of the male without castration.

TESTICULAR HORMONES. Hormones produced by testicular tissues are known as androgens. They are also present in urine. Although they are termed male sex hormones, they have certain estrogenic properties. So far as the male is concerned, these hormones serve to bring about



testosterone



androsterone

normal development of the male reproductive organs and to preserve and maintain secondary male characteristics. Two typical androgens are testosterone and androsterone. From their structures it can be seen that the "male" and "female" steroids are closely related chemically.

MILK

Mammary Glands

The mammary gland differs from the organs described above in that the secretion is used for food for the young mammal rather than for regulation of metabolism. Mammary glands develop in the young female mammal at puberty with proliferation of tubules and development of alveoli. Liberation of the ovarian hormones initiates this growth. Milk is secreted by the alveoli of the mammary gland after proper stimulation by a series of hormones immediately after parturition. Prolactin, a pituitary hormone (page 319), is the chief hormone involved in the initiation of the process. Oxytocin plays a role in the release of milk from the lactating gland through its action on smooth muscles, and adrenal hormones are thought to function although the mechanism is not defined.

Composition of Milk

Milk is the principal food of the young mammal. It is a white, opaque fluid containing 64 to 88 per cent water, 1.5 to 10.3 per cent protein, 3.5 to 19.7 per cent fat, 4.5 to 7.2 per cent lactose, and 0.2 to 1.5 per cent minerals, depending on the species of origin (page 416). The white color of milk is due to finely dispersed fat globules, coated with a film of protein and phospholipide.

Milk contains three chief proteins, casein, lactalbumin, and lactoglobulin, with casein constituting approximately 80 per cent of the total protein. Casein is a nutritionally complete protein in that it is rich in all the essential amino acids and therefore has been used as a standard in most experimental protein-feeding trials. It is a phosphoprotein with an isoelectric point of pH 4.6, where it is water insoluble. In milk, casein is probably present as the calcium salt. Casein has found extensive use as a food in cheeses, as an industrial protein with a variety of functions in filaments and films, and as an adhesive or filler.

Milk fat is a mixture of triglycerides containing, as a rule, about two-thirds saturated fatty acids from 4 to 16 carbons in length and approximately one-third oleic acid by weight. Species, age, and state of nutrition are factors which affect the quantity of fat produced and the fatty acid distribution in the triglycerides.

The ash of milk contains all the elements required for growth of the young mammal but not always in optimal quantities. Iron and copper supplementation is sometimes required in the growing young to prevent anemia. Milk contains most of the vitamins in greater or smaller amounts. Generally it is deficient in C and D, although there are great species variations. The diet of the lactating mammal is also a factor in vitamin content.

The early secretion of the mammary gland, called colostrum, is quite different from the milk produced later. Colostrum is relatively high in protein and low in carbohydrates, fats, and ash. The globulin content of colostrum is relatively high, as is the antibody titer. The blood of the newborn mammal has a very low antibody titer, which means that it is susceptible to pathogenic organisms. The antibodies of colostrum milk are quickly absorbed, but the process of absorption is poorly understood. In the older mammal, transfer of such large particles does not occur so extensively. Colostrum may be a waste product which follows regeneration of the secretory tissues of the mammary glands. As soon as lactation ceases, the globulin fraction of the fluids of the glands increases and casein decreases. The globulin content of such tissue remains high until the next lactation period. The antibodies of colostrum milk may be produced to protect mammary tissue during the interim between lactation periods.

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Vitamins

14

The role of foods in the life, growth, and health of animals has been a subject of philosophical speculation at least since the classical period of Greece. Suitable foodstuffs were believed to contain a vital substance without which materials were unsatisfactory as foods. This theory persisted until perhaps 1827, when Prout suggested that mammals required principles described in terms now replaced with protein, carbohydrate, and fat.

For decades it was believed that these three classes of materials were the only essential nutrients. Moreover, it was further considered that all proteins were nutritionally alike and that the various carbohydrates and fats were quite equivalent. Therefore, it was believed that the best diets for animals and men were those highest in the three groups of compounds. As a logical extension of this thinking, much effort was expended in the analysis of foods from this point of view.

In 1871 the siege of Paris imposed great hardship on the inhabitants, and the supply of milk to the city was blocked. Since the proportions of protein, carbohydrate, and fats in milk were known, an emulsion of corresponding composition was proposed as a substitute in the diets of infants and children. As might be anticipated now, the results were tragic. Later that year the chemist Dumas wrote an account of the dietary problems arising from the siege and the imitation milk and stated that ". . . no conscientious chemist can assert that the analysis of milk has made known the products necessary to life which that aliment contains."

Although Dumas clearly realized the critical importance of unknown factors, little concern was given to the new concept. However, in 1880

Lunin showed that mice remained healthy on milk alone but died when fed a mixture of casein, lactose, milk fat, and milk ash in the same proportions as in milk itself. On the basis of these observations he wrote, ". . . other substances indispensable for nutrition must be present in milk besides casein, lactose, fat, and salts." This conclusion was the culmination of a series of feeding experiments in which animals failed on diets of purified proteins, carbohydrates, and fats. Again the new idea did not gain general acceptance.

Actually the disease called scurvy had been recognized in armies and on shipboard since antiquity. At least as early as 1601 oranges and lemons were officially recognized as preventives of the symptoms. Lind published a treatise on scurvy in 1753 in which the cure of severe cases within 6 days was described, using fresh fruits and vegetables. He based his opinion on convincing experiments conducted by himself on sailors.

In spite of many similar statements, it was apparently not until Dumas and Lunin that unknown factors were suspected as essential to complete nutrition. Even the first systematic prevention and cure of beriberi were mistakenly attributed in 1882 to an increase in dietary protein. This disease, mentioned first in China in 2600 B.C., was eliminated from the Japanese navy by Takaki, Director-General of the Navy Medical Service, who supplemented the diet of polished rice with increased allowances of vegetables and meat and substituted barley for part of the rice. Although Takaki did not realize the ultimate cause of beriberi, he understood correctly that the disease was caused by an unbalanced diet.

Still another disease, rickets, was attributed to faulty diets, and in 1838 Guerin produced it experimentally in puppies to prove the connection.

The prevailing explanation of these cures is well represented by observations made at the end of the nineteenth century on experimental beriberi in chickens. Hens fed rice without bran developed symptoms that were cured when the bran was added. Eijkman continued this work, observing that the antiberiberi factor could be readily extracted and that it was small enough to be dialyzable. However, he regarded the factor as an antidote for an organism he thought occurred in rice and caused the disease.

Again the idea of a special nutritional factor was proposed, this time in 1901 by Grijns, who suggested that beriberi appeared because the diet "lacked certain substances of importance in the metabolism of the central nervous system." Finally, after isolation of the factor in 1912 by Funk, nutritionists at last adopted the view that special substances were required in small amounts by animals. At this time Funk coined the term *vitamine* (the *e* is now omitted) to describe his vital nutritional factor, which had the chemical properties of an amine. Now the vitamins include several chemically unrelated groups of compounds required for the well-being and normal functioning of various animal species.

Even when nutritionists fully realized the possible occurrence of essential factors, little progress was made at first in relating particular physiological symptoms to certain experimental diets. In 1906 Hopkins concluded that not only beriberi but scurvy and rickets as well were caused by nutritional deficiencies, but a solid basis for general belief came only after Funk's isolation of the factor preventing beriberi.

Finally an idea of the complexity of the problem appeared when McCollum and Davis (1913 to 1915) found that a combination of factors was required. Rats failed when fed wheat alone or wheat supplemented with a purified protein like casein, egg fat or butter, or a salt mixture. Even when any three were combined rats did not grow and reproduce normally. Yet they did so when all four materials were used, indicating that at least four factors were essential. The inorganic ions of the salt mixture and amino acids of the casein were needed to supplement those of wheat. In addition, the wheat contained water-soluble factors which came to be called vitamin B. The fat-soluble materials, called vitamin A, came from butter or egg. Both vitamins turned out to be mixtures of materials.

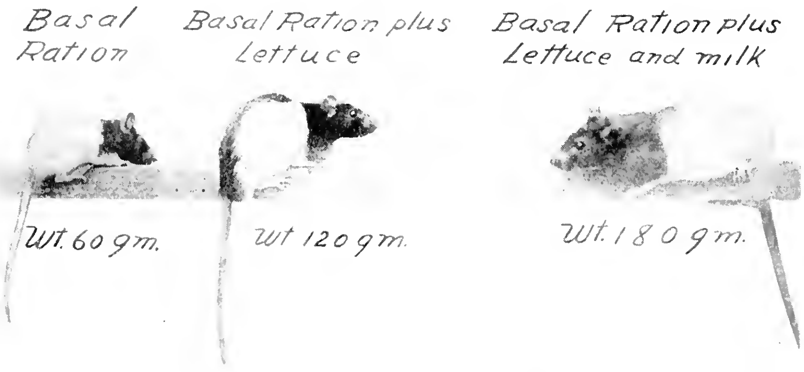


FIGURE 14-1. These rats were of the same age and received the same basal diet with the exception of the supplements noted. This experiment emphasizes the importance of milk and leafy foods in the diet.

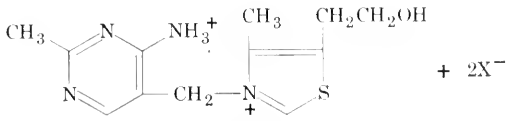
The chemical diversity of the vitamins makes classification difficult. As a compromise they are grouped into water-soluble and fat-soluble vitamins and further associated where possible into the various chemically similar substances fulfilling a single nutritional need in one or more animals. The discovery and study of all these factors represent one of the historically interesting phases of science. See Figure 14–1 for an example of the effect of dietary vitamins on growth.

THIAMINE

Vitamin B turned out to be a number of essential water-soluble nutrients. Those examples that have been characterized up to now bear individual names assigned more or less at random. The first to be isolated was the antiberiberi factor now called vitamin B_1 or thiamine.

Chemistry

After thiamine was isolated, structural and synthetic studies culminated in 1936 in the complete structural formula and synthesis of the vitamin. By 1957 the compound was commercially available for therapeutic purposes, enrichment of foods, vitamin supplements, etc., at $10/kg., which amounts to about 500,000 daily allowances.

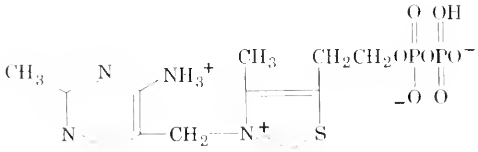


thiamine (vitamin B_1)

At physiological pH values thiamine occurs as a cation with the structure shown, associated with two anions, usually chloride or nitrate. The size and complexity of this molecule account for the difficulties encountered and the time consumed in working out the structure. Its relatively low price is a tribute to the skill of the chemical industry.

Function

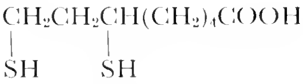
This vitamin is an important portion of the coenzyme called cocarboxylase, or more recently thiamine pyrophosphate, written thus:



thiamine pyrophosphate (cocarboxylase)

This cofactor is required in several enzymatic processes for the addition or removal of carboxyl groups. One of the principal reactions known to require this coenzyme is the decarboxylation of pyruvate with pyruvic dehydrogenase to form carbon dioxide and acetyl coenzyme A. This process is shown on page 171 as the connecting link between glycolysis and the tricarboxylic acid cycle. Reactions catalyzed by either transketolase or transaldolase also require thiamine pyrophosphate. For example, see pages 213 and 216.

The exact mechanism by which this coenzyme functions has not been conclusively demonstrated. The most prevalent opinion holds that it forms a complex with both the enzyme and the substrate. It is known that the hydrogen attached to the carbon atom between the nitrogen and the sulfur is very labile. As a result it has been suggested that the carbonyl group of α-keto acids attacks this position and undergoes decarboxylation. The next step postulated involves transfer of the acyl group to lipoic acid and finally, using the enzyme thiol transacetylase, to coenzyme A.



$$\underset{\displaystyle SH}{CH_2}CH_2\underset{\displaystyle SH}{CH}(CH_2)_4COOH$$

lipoic (thioctic) acid

It is obvious that increased knowledge of metabolism is likely to bring out additional roles played by thiamine or its derivatives. As a matter of fact one special derivative has been reported in the bacterium *Escherichia coli*, where thiamine pyrophosphate was thought to be coupled to lipoic acid. The resulting complex was then active in the enzymatic decarboxylation of pyruvate. Other workers feel that the thiamine pyrophosphate and lipoic acid are indeed both essential but that the postulated amide bond between them does not exist. Thus a complete mechanism of this key reaction is not available.

Requirements

All animals except the ruminants require a regular supply of thiamine in their diets. Ruminants need thiamine too, but the bacterial flora of their rumens synthesize and liberate enough of the vitamin to supply the animals' needs. Minimal requirements for other species cannot be set accurately because the values are known to vary with individual body weight, caloric intake, composition of the diet, and unknown factors.

As a result the dietary intakes usually recommended allow safety factors and provide enough thiamine to prevent the appearance of deficiency symptoms in normal individuals. For healthy human beings the daily allowance is put at 0.5 mg. of thiamine per 1,000 cal. consumed. On this basis, the total intake should range from 0.3 to 0.5 mg. for infants and to about 2.0 mg. for very active individuals of larger-than-average size.

Although birds are very susceptible to a lack of thiamine in the diet, the poultry farmer is not required to give much thought to the thiamine content of his poultry rations. Good poultry rations usually contain more thiamine than the birds require. Starting chicks, up to 8 weeks of age, should receive about 0.9 mg. of thiamine per lb. of feed. No requirements or allowances have been established for older birds.

The daily allowances recommended for swine range from 1.4 mg. of thiamine for pigs weighing 50 lb. to 4.2 mg. for hogs of 250 lb. Lactating sows and breeding swine should receive about 6.3 mg./day.

Thiamine is quite widely distributed in foods and feed stuffs, but very few of our common foods are rich sources of this vitamin. The richest natural sources are yeast, cereal grains, and pork. Potatoes and other vegetables play an important role as a source of thiamine in spite of the fact that these foods are not rich in this factor. White flour, like polished rice, is a poor source of thiamine because most of the vitamin is lost in the refining process. Wheat bran is a fairly rich source of vitamin B_1. Raw milk is not a good source of thiamine, and the normal content may be reduced appreciably by pasteurization. Yet milk is important in America as a thiamine source because of the great volumes consumed. The outer layers of most seeds and most animal tissues are rather high in thiamine and play important roles in balanced diets.

It is now customary to enrich white bread to replace the vitamin lost in milling white flour. This extra supply supplements diets that nutritional surveys show are marginal even in this country and Europe. Nevertheless, cases of vitamin B_1 deficiency are rare in the United States except in chronic alcoholism, where it is sometimes correlated with improper eating habits or lack of sufficient food. In the Far East, use of polished rice as the dietary staple leads to great numbers of cases of beriberi. Educational programs have improved the situation, but thousands of cases undoubtedly still occur in remote areas.

Thiamine is lost in varying amounts when vegetables are cooked. These losses vary according to cooking temperatures, types of food, and the amount of cooking water thrown away before the food is served. Ordinarily the loss of thiamine during cooking should not exceed 25 per cent if foods are cooked properly in a minimum of water. Canning processes are not particularly destructive.

In many cases thiamine losses in vegetables may be greater during harvesting, handling, and storage than during actual processing in the cannery. Under normal conditions, however, blanching, a short heat treatment, seems to be the most important single factor affecting vitamin losses in canned foods. Even canned goods must be stored at relatively cool temperatures (80°F. or less) to prevent thiamine losses during storage. Freezing *per se* has little or no effect on thiamine. The greatest losses of thiamine in frozen foods occur before the freezing process or during the subsequent thawing and cooking. Rapid dehydration of foodstuffs at relatively low temperatures may preserve over 75 per cent of the original thiamine content, whereas prolonged exposure at elevated temperatures destroys much of the vitamin.

Deficiency

Since diets deficient in thiamine are often low in other essential factors as well, uncomplicated cases of thiamine deficiency are seldom encountered. Moreover, the symptoms are quite general, thus making diagnosis difficult. Very complex pathologies may develop, differing considerably from one individual to another. The general effect, however, is development of abnormal muscle and nerve responses in various parts of the body.

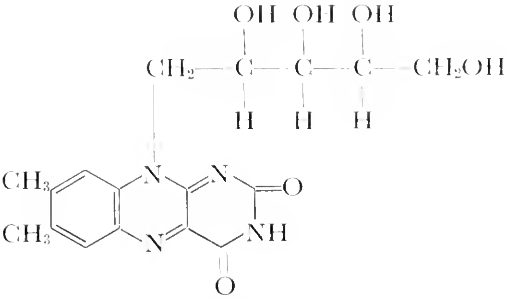
As expected, the severity of symptoms corresponds to the extent and duration of the deficiency. Loss of appetite and weight, weakness, shortness of breath, palpitation of the heart, and muscle soreness, especially of the lower parts of the legs, are early indications. Progress of the disease leads to mild anesthesia, mental confusion, muscle weakness and partial paralysis, loss of reflex activity, peripheral neuritis, brain lesions, and cardiac malfunction. Ultimately, the victim becomes helpless or insane and succumbs, often to heart failure. Severe edema sometimes accompanies the other manifestations. Deficient birds lose control of their neck muscles, and their heads tip characteristically over on their backs.

These various symptoms presumably result from the metabolic disturbances accompanying the lack or deficiency of thiamine pyrophosphate. Oxidative metabolism is greatly impaired without the coenzyme, and both pyruvic and lactic acids accumulate in large amounts since utilization of pyruvate is thus blocked. Nerve cells seem to be especially dependent on the utilization of carbohydrates, and for that reason a thiamine deficiency is more serious in nerve tissues, leading quickly to pronounced alterations in nerve function.

RIBOFLAVIN

Chemistry

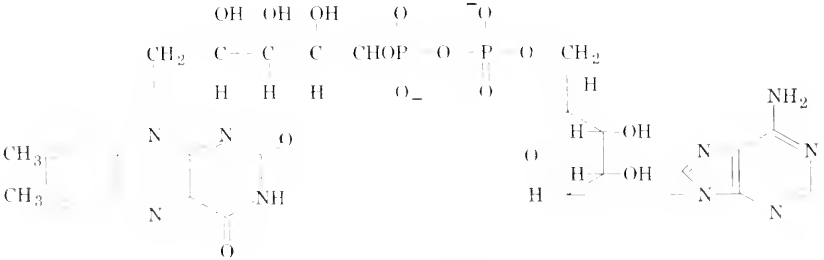
Riboflavin, an essential substance also called vitamin B_2, was first isolated in 1879 from milk. Yet its importance was not realized until after extensive studies in 1933 and 1934 showed that the compound promoted the growth of rats. The accepted structure,



riboflavin (vitamin B_2)

is composed of the lower isoalloxazine portion attached to the group related to D-ribose and called the D-ribityl group. Although complex, this molecule has been synthesized and in 1957 was available in pharmaceutical grades at $40/kg.

Riboflavin functions in the animal body in the form of prosthetic groups (for proteins) called flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The former differs from riboflavin itself in having a phosphate attached to the end hydroxyl of the ribityl group. Flavin adenine dinucleotide is written thus:

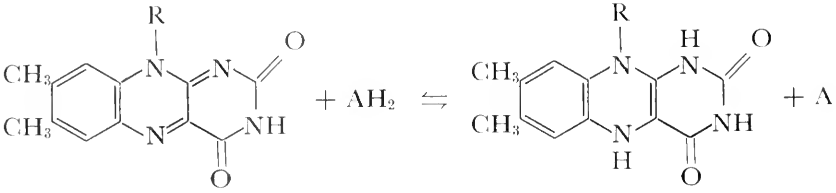


flavin adenine dinucleotide (FAD)

It is at once apparent that the right half of this molecule is like that of the coenzyme DPN^+ (page 140).

Function

Riboflavin serves by way of one of the above prosthetic groups as a hydrogen-electron carrier, as mentioned earlier. The isoalloxazine portion of the molecule is subject to reversible oxidation and reduction according to the reaction shown,



where AH_2 represents a suitable reducing agent and R the rest of the structure for either FMN or FAD. The R group helps contribute a physiologically suitable oxidation-reduction potential and provides for attachment to the apoenzyme component.

It is now known that the various flavoproteins are also metalloproteins containing either Mo^{++}, Cu^{++}, or Fe^{++}. These ions are important to the activity of the enzymes since their removal blocks the ordinary function. Probably the prosthetic group accepts the electrons and transfers them to the metal ion, which in turn transfers electrons to the next component of the chain.

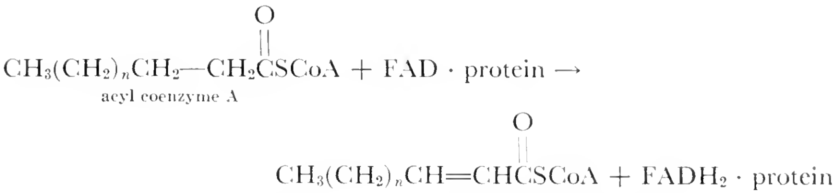
$$\begin{aligned}
&2H_3O^+ + (2e) + \text{FAD} \cdot Fe_2^{+++}\ \text{protein} \longrightarrow \\
&\qquad\qquad 2H_2O + \text{FADH}_2 \cdot Fe_2^{+++}\ \text{protein} \\
&\qquad\qquad\qquad\qquad \downarrow \\
&\text{FAD} \cdot Fe_2^{+++}\ \text{protein} + (2e) \longleftarrow \text{FAD} \cdot Fe_2^{++}\ \text{protein} + 2H_3O^+
\end{aligned}$$

In this system the electrons come in one end from a reduced substrate and are accepted at the other by an oxidized substrate. It is believed that in at least one flavoprotein there are four iron ions for each FAD instead of the two written here for the sake of convenience.

The particular electron-transfer system outlined on page 172 involves the flavoprotein called cytochrome reductase and functions by the above mechanism. DPNH supplies the electrons to the cytochrome reductase, which transfers them to cytochrome on the way to molecular oxygen. Although this pathway may not represent the only one in animals, it is certainly the major one. Hence, the importance of riboflavin in the diet becomes evident for all those animals unable to synthesize it.

In addition to cytochrome reductase, there are other important flavoenzymes. Xanthine oxidase, for example, catalyzes the oxidation of a number of different aldehydes as well as purines and pyrimidines like xanthine. This enzyme is widespread and probably functions in the formation of essential protoplasmic components and in the metabolic conversion of purines and pyrimidines into compounds suitable for excretion. Xanthine oxidase contains iron and molybdenum with the FAD group, and all are essential for its action.

During the metabolism of fats, various acyl coenzyme A oxidations occur. These require flavoproteins which catalyze the removal of hydrogen atoms from the α and β carbons of the acyl group. This particular reaction



$$\underset{\text{acyl coenzyme A}}{CH_3(CH_2)_nCH_2{-}CH_2\overset{\overset{O}{\|}}{C}SCoA} + FAD \cdot \text{protein} \rightarrow$$

$$CH_3(CH_2)_nCH{=}CH\overset{\overset{O}{\|}}{C}SCoA + FADH_2 \cdot \text{protein}$$

may play only a catabolic role by transferring electrons to the cytochrome system by way of a "cytochrome reducing factor," also a flavoprotein but not yet well characterized. A variety of other flavoproteins participate in still other oxidations of importance, particularly of amino acids. Specialized discussions may be consulted for details.

Requirements

Recommended daily allowances for optimal nutrition range from 1.5 to 3.0 mg., depending on age, sex, and physical activity. The highest allowance (3.0 mg.) is recommended for pregnant and lactating women.

Recommended allowances for domestic poultry are expressed in terms of milligrams of riboflavin per pound of feed rather than on the basis of daily intake. Starting chicks should receive feed containing about 1.6 mg. of riboflavin per lb., and the allowances for laying and breeding hens are 0.9 and 1.3 mg., respectively. A ration containing sufficient riboflavin for egg production may not be sufficiently rich in this vitamin to ensure maximal hatchability. The supply of riboflavin in the egg must be high enough to allow full development and growth of the chick. Otherwise it dies of a deficiency before hatching.

A mutant strain of White Leghorns is now available with a genetic incapacity in the hens preventing transfer of sufficient riboflavin to the egg for hatching. Fertilized eggs are otherwise normal, since injection of riboflavin leads to hatching of chicks normal except for their inherited tendency to lay eggs deficient in the vitamin.

Turkeys appear to have a higher requirement than chickens. Poults and breeders require, respectively, 2 and 1.6 mg. of riboflavin per lb. of feed.

Daily riboflavin allowances for growing swine range from 2.1 mg. (50 lb. live weight) to 6.3 mg. (250 lb. live weight). No allowances have been recommended for breeding, pregnant, and lactating animals. The tentative allowance for horses is 2 mg. per 100 lb. of body weight. Since bacteria synthesize riboflavin in the rumen in quantity, ruminants do not need a dietary source of this vitamin.

Inasmuch as riboflavin is a factor in many metabolic processes, it occurs in all metabolically active plant and animal tissues. The best dietary sources are yeast, liver, kidney, milk, eggs, fish, oysters, and muscle tissues. Alfalfa meals are relatively good and cereal grains are fair but not rich sources. Fermentation residues from alcohol manufacture probably offer the richest large supplies.

Deficiency

Lack of riboflavin leads to bloodshot eyes, a typical dermatitis, and loss of hair as early symptoms in human beings and animals. Human beings later develop facial lesions, especially of the lips, and cracks

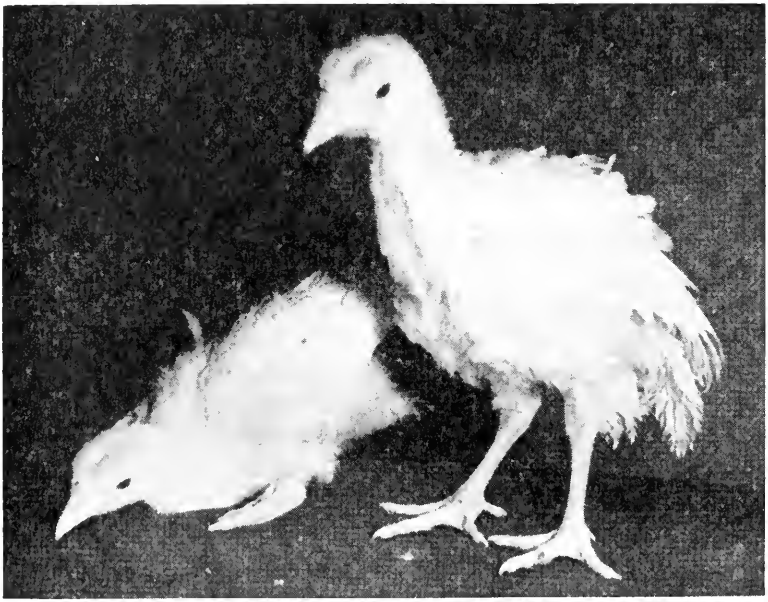


FIGURE 14-2. Effects of the absence and the presence of riboflavin in the ration of turkey poults. Note retarded growth and prostrated condition (leg paralysis and curled toes) of the poult which received the riboflavin-deficient ration in contrast to the poult which received the same ration supplemented with crystalline riboflavin. (Courtesy of Dr. R. V. Boucher.)

occur at the corners of the mouth. The disease called pellagra is often partly due to riboflavin deficiency and carries these same symptoms. It is also common for patients to become sensitive to light and suffer from blurred vision. These symptoms usually disappear readily when riboflavin is administered.

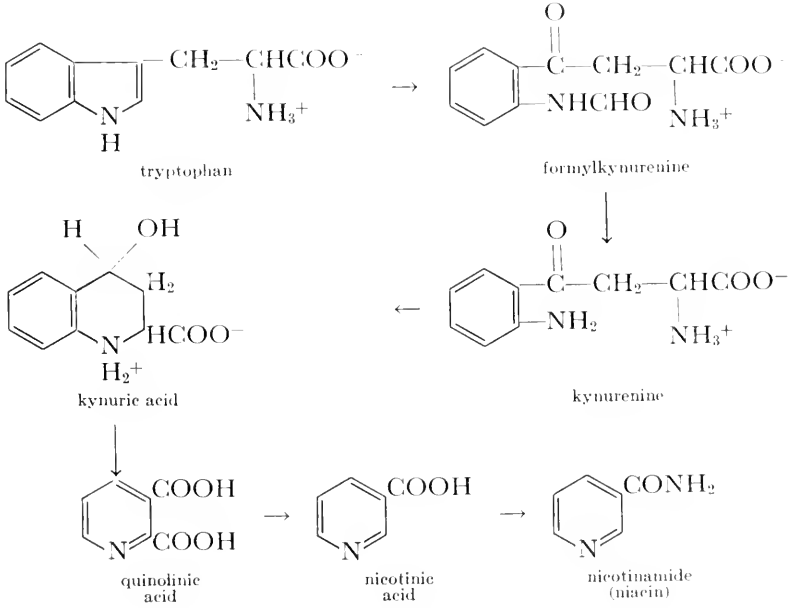
Since the substance is essential to so many metabolic reactions, severe and prolonged deficiency leads to serious pathologies, including nerve degeneration, weakness, paralysis, cataracts of the eyes, impaired reproduction, malformations of limbs, and respiratory failure. See Figure 14–2. Obviously, when too extensive, such changes may not be reversed by vitamin therapy. Deficiency of riboflavin alone is rather rare. This fact and a marked lack of specificity in symptoms, complicated with other pathological states, make diagnosis of deficiency quite difficult.

NICOTINAMIDE

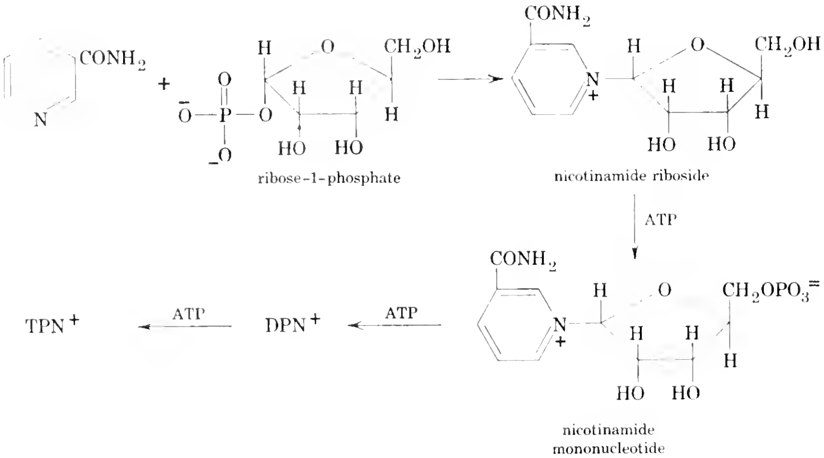
Chemistry

This substance has been known since 1867 as an oxidation product of nicotine. Moreover, the disease pellagra was recognized by 1735 and postulated to be dietary by 1912. Yet it was not until 1937 that the antipellagra factor was shown to be nicotinamide.

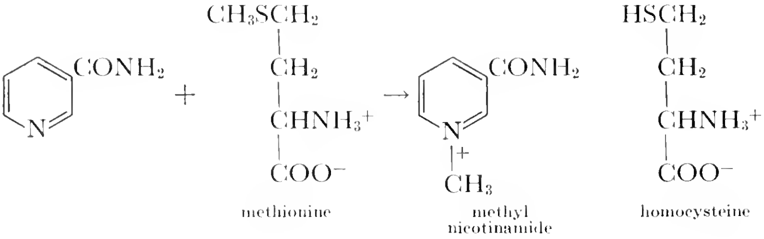
Almost all organisms synthesize this essential factor, probably by the following route:



Some of these reactions probably consist of more than one step. The nicotinamide is then converted into DPN+ and TPN+. See pages 140 and 170 for the structures of these coenzymes, which are the only known forms in which the nicotinamide functions in cells.



More is known about the metabolism of nicotinamide than about that of the other vitamins. This substance is methylated before its excretion by man in urine.



Other transformations are known to occur in plants, birds, and dogs. These details may be obtained from reviews covering the biochemistry of nicotinamide and nicotinic acid.

Function

As an essential part of both DPN+ and TPN+, nicotinamide participates in a great variety of important oxidation-reduction processes. No other role is known. The two coenzymes are quite indispensable in all known metabolic systems where they serve as hydrogen-electron carriers in many enzymatic reactions. See particularly pages 172 and 222. It will be recalled that the major terminal oxidation of higher animals and most plants involves coupling of the DPN$^+$-flavin-cytochrome-protein systems.

Requirements

It is even more difficult to arrive at daily allowances for niacin than for thiamine and riboflavin. Most species synthesize nicotinamide, but the quantity depends upon the diet. Animals can use nicotinic acid as well as the amide. Neither is essential under favorable dietary conditions if the supply of tryptophan is adequate. Thus proteins rich in this amino acid are helpful but can prove inadequate compared to free tryptophan. Apparently dietary tryptophan is not converted to niacin extensively enough in all cases.

Recommended daily allowances range from 5 mg. for infants to 10 to 20 mg. for adults, depending on age, sex, and activity. These intakes are believed to provide a safety factor for the uncertainties in the effect of diets and the possible activity of intestinal bacteria. Starting chicks should receive about 8 mg. of nicotinic acid per lb. of feed, but no recommendations have been made for laying and breeding hens. Daily nicotinic acid allowances for growing pigs range from 7 to 21 mg., depending on age and weight. Ruminating animals do not require dietary niacin.

Meats, fish, and yeast are perhaps the best sources of the factor. Green vegetables, milk, and eggs are effective, the last two because of the tryptophan present, since they are almost devoid of nicotinamide. Corn is not a good source unless steeped in alkali first. This treatment yields substances converted to niacin. Other cereal grains are not satisfactory when the outer layers are removed. White flour is low in nicotinamide, so it is customary to enrich it for bread, using synthetic material at about $10/kg. Since the vitamin is stable to heating, oxidation, and the pH values encountered in all foods, processing does no harm except in cases of physical removal.

Deficiency

Lack of nicotinamide and its substitutes, nicotinic acid and tryptophan, leads to pellagra, often in complicated form with other deficiencies present. Digestive disturbances accompany the appearance of inflamed areas of the skin, predominantly of the face and hands. Later the skin becomes scaly, rough, and pigmented brown; finally open sores develop. The tongue swells and ulcerates, weight is lost rapidly, and neural symptoms appear leading to insanity and death. Before brain lesions develop and nerve injury becomes acute, treatment with niacin is rapidly and rather completely effective. The connection between the metabolic functions of the vitamin and the appearance of the characteristic symptoms is not known.

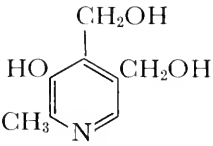
Pellagra is limited primarily to the lowest economic groups in areas where cornmeal is a staple food. It has been most serious in the southern parts of the United States and Italy and in Egypt. In Mexico a similar wide use of corn is not a predisposing factor because of the alkali treatment in the processing method employed there. In the United States in 1917, 170,000 cases of pellagra were reported, and many mild cases were undoubtedly never diagnosed. Even by 1927 there were still over 120,000 cases, with more than 21,000 known deaths in 1928 to 1930. From 1911 to 1916 there were more deaths from pellagra than from tuberculosis or malaria, the leading infectious diseases. Incredible as it may seem, over 600 deaths were attributed to pellagra in 1948, in a period of great national prosperity and general economic well-being. It is encouraging that the death rate is declining rapidly.

Most American cases are due to diets consisting mainly of cornmeal, salt pork, and molasses with an insufficiency of the meat. The disease is associated with insanity as both a cause and a result. In some countries pellagra is common among prisoners given very poor food. Occasionally it appears in individuals with good diets but with poor absorption of the vitamin. Chronic alcoholism also produces some fatal cases.

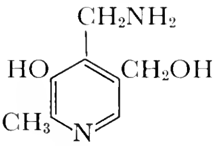
VITAMIN B$_6$

Chemistry

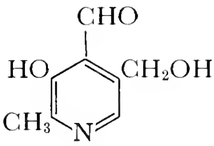
This essential factor turned up in connection with studies on pellagra, where it sometimes occurs as a part of that multiple deficiency. Although microorganisms may be specific for the chemical form of the vitamin they can utilize, animals thrive on any one of four derivatives.



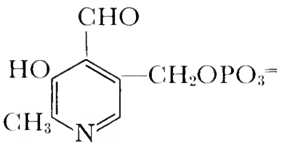
pyridoxine



pyridoxamine



pyridoxal



pyridoxalphosphate

It is believed that the first two are converted to pyridoxal, which is then phosphorylated to form the real coenzyme, pyridoxalphosphate. All forms are available commercially, but the coenzyme itself is still \$50/100 mg. Only pyridoxine is made on a sufficient synthetic scale for use in vitamin supplements at \$295/kg. of the hydrochloride.

Function

In so far as is known, only pyridoxalphosphate participates in metabolism. In this form vitamin B_6 is both universally distributed and involved in a variety of reactions. Many of these are important in the metabolism of amino acids serving in decarboxylations, oxidations, transaminations, racemizations, and syntheses, especially of tryptophan.

A complex and detailed mechanism of action has been worked out for pyridoxalphosphate in most of its roles. Since modern theories of advanced organic chemistry are fundamental to this outline, it is not presented here. Reviews should be consulted for a full presentation.

Requirements

Daily requirements depend upon the intake of protein and amino acids, especially tryptophan. The intestinal flora affects the supply. Hence it is impossible to estimate reliably an allowance for man in the absence of extensive dietary studies with human beings. Deficiencies may occur in conjunction with those of any of the other vitamins already discussed. Rats require about 10 mg. per day, whereas chicks need about 1.6 mg/lb. of feed.

Vitamin B_6 is usually associated in nature with the other members of the B group, including niacin. It is relatively abundant in grains and seeds, egg yolk, yeast, and meat. Corn is a good source in contrast to its low nicotinamide content.

Deficiency

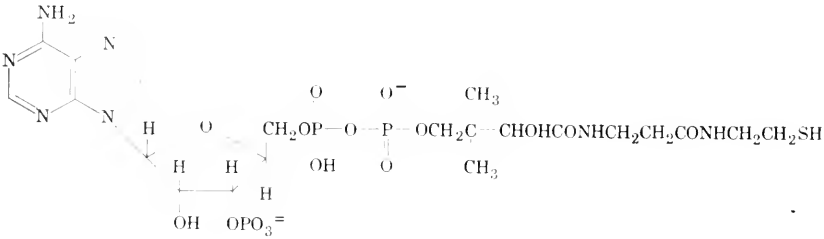
Natural uncomplicated deficiencies of vitamin B_6 are unknown in man. Experimental deficiencies in rats and pigs lead to loss of hair, dermatitis, poor growth, loss of weight, anemias, and convulsions. Degeneration of spinal and peripheral nerves occurs. Dairy cattle sometimes develop similar symptoms spontaneously. Administration of the vitamin reduces these disturbances quickly during early stages of the disease.

PANTOTHENIC ACID

This member of the B group of vitamins is the amide of a derivative of butyric acid, called pantoic acid, and β-alanine. Its only known role is as part of coenzyme A, whose structure is as shown. The left portion of the molecule is a phosphorylated ADP, and the right portion is β-mercaptoethylamine, probably formed by decarboxylation of cysteine. Coenzyme A is required in decarboxylations like those of pyruvate and α-ketoglutarate in the tricarboxylic acid cycle. It is involved in the metabolism of hydrocarbon side chains, particularly those of amino acids and fatty acids. See Chapters 19 and 20. This coenzyme is quite essential to life.

$$HOCH_2-\underset{CH_3}{\overset{CH_3}{C}}-\underset{OH}{CH}-CONHCH_2CH_2COOH$$

pantothenic acid



coenzyme A

Pantothenic acid and coenzyme A are synthesized by green plants and by many microorganisms. Animals cannot make pantothenic acid, so this substance must be present in the diet to serve as a starting point for coenzyme A. A mixture of β-alanine and pantoic acid will not suffice. Somewhat surprisingly, pantothenic acid is not lost by hydrolysis in the gastrointestinal tracts of animals.

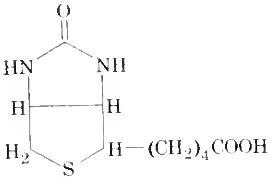
The daily requirement of man is unknown. Recommendations of 2.5 to 5.0 mg./lb. of feed have been made for poultry with the lower figure for chickens and the higher value for ducks and turkeys.

Most fruits and vegetables are rather low in pantothenic acid, but yeast, meat, eggs, and milk are good sources. Pharmacological grades of the calcium salt sell for $45/kg. and are used in enriching white flours.

Human deficiency states cured with pantothenic acid are common in certain areas in India. Sore tongue, anemia, foot irritations, and fatal necrosis of the adrenal cortex are symptomatic. Some cases occur in hospitals and result from special diets. Characteristic loss of hair pigments in particular areas occurs in experimental rats, pigs, and dogs. Dermatitis, growth failure, nose bleed, and failure of the adrenal cortex are additional symptoms.

BIOTIN

This vitamin is also a member of the so-called B complex and is assigned the following structure:



biotin

In many biological materials it occurs as the amide of the ε-amino group of lysine. Biotin is synthesized commercially and included in many vitamin supplements.

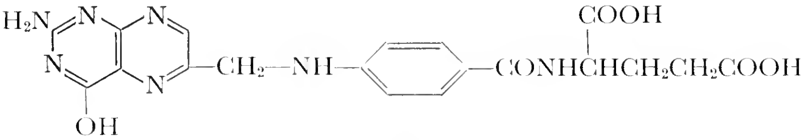
Nothing definite is known of the metabolic functions of this vitamin, which plays roles as yet unspecified in the carboxylation and decarboxylation of certain acids of physiological importance. Nevertheless, this substance deserves its inclusion with the other vitamins since it is universally distributed and deficiencies can be induced.

By extrapolation from studies with rats, the daily human requirement is about 10 mg., one of the lower allowances. Biotin is synthesized in the rumen, making it unnecessary in the diets of ruminants. Indeed, widespread synthesis by intestinal flora makes it difficult to produce experimental biotin deficiencies. Chicks and turkey poults are believed to require 45 to 70 mg. of biotin per lb. of ration.

Biotin occurs particularly in egg yolks, yeast, meat, milk, vegetables, and seeds. Although deficiencies are rare in nature, they can be induced readily by feeding a diet containing raw egg white as the sole or major source of protein. Egg white contains a particular protein called avidin which inactivates biotin by removing it from an otherwise complete diet. If the intestinal flora is then controlled with a suitable antibiotic, deficiency symptoms develop. Dermatitis, loss of hair and weight, edema, and skin lesions appear, with changes in posture and gait. Death may result. Heating egg white destroys the avidin and prevents the so-called egg-white injury.

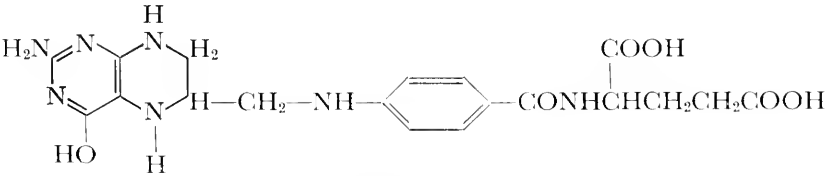
FOLIC ACID

This vitamin has been known by a variety of names, most of which are no longer used. The structure confirmed by synthesis is shown.



folic acid

An amide of *p*-aminobenzoic acid and glutamic acid is attached to the left-hand portion of the molecule called a pterin. Some bacterial species require a supply of *p*-aminobenzoic acid, which is needed in turn in forming folic acid. There is limited indirect evidence that *p*-aminobenzoic acid may play other roles in certain microorganisms. There is a series of these folic acid vitamins differing only in the number of attached glutamic acids, which may range to at least seven units. Folic acid itself is probably reduced to tetrahydrofolic acid, which actually participates in the transfer of one carbon unit in various metabolic processes, for example, serine-glycine interconversions. (See page 466.)



tetrahydrofolic acid

While this vitamin (folic acid) is certainly indispensable, its wide distribution makes deficiencies somewhat rare. Moreover, rapid synthesis by intestinal bacteria provides an additional supply, making it difficult and almost unnecessary to assign daily allowances. None have been fixed as yet. Folic acid is relatively abundant in leafy vegetables, beef, and many seeds and grains, especially wheat.

This factor is useful in treating certain abnormalities of the blood, primarily macrocytic anemias with reduced numbers and oversized red cells. An anemia of this kind is sometimes associated with pellagra, pregnancy, and the tropical disease called sprue. During World War II wide use of sulfa drugs to prevent infection after injury also eliminated the normal intestinal bacteria that synthesize folic acid. In some areas of the Pacific the available diets were low in folic acid, and deficiency anemias developed in hospitals before the role and distribution of the vitamin were as well known as they are today.

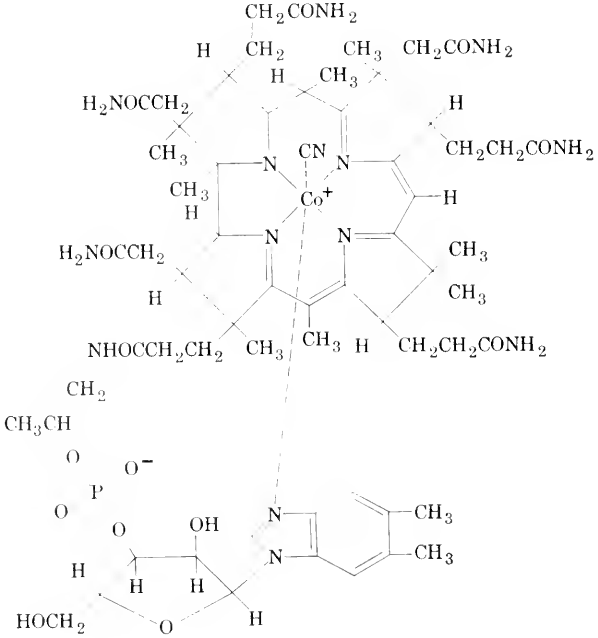
Folic acid prevents and cures characteristic anemias in rats, chicks, and monkeys. It is essential for lactation in rats and hatchability of eggs as well as for normal growth of chicks, turkeys, and guinea pigs. Hence this vitamin is probably essential for all the higher animals at least.

Sulfa drugs prevent growth of many bacteria by acting as antagonists for *p*-aminobenzoic acid and blocking the formation of folic acid. Thus they can be used to prevent or reduce infections before the animal as a whole develops serious deficiency symptoms. Analogues are also known for folic acid itself. Although these compounds are rather toxic for general clinical application, one has been used to produce temporary remissions of acute leukemia. This drug, aminopterin, also causes partial recessions of certain experimental tumors but is not particularly effective on human cancers.

VITAMIN B_{12}

This rather recently isolated member of the water-soluble group of vitamins is also called cobalamine and is unique among vitamins in containing a metal atom. Although not proved in all respects, the presently accepted structure is based on extensive physical and chemical data. The two bonds represented by dotted lines lie along the axis perpendicular to the plane of the paper. This extremely complicated molecule has not been synthesized. For clinical and experimental use it is isolated at a cost of 25¢/μg. from certain mold cultures grown in the production of antibiotics. Related compounds have been isolated and studied to a limited extent.

Vitamin B_{12} is very low in plants which may not make appreciable use of it. It apparently occurs in all animal tissues. Since animals are not known to synthesize it, they must depend for their supplies on other animals or on microorganisms. Strict vegetarians may develop marginal deficiencies if their intestinal flora are disturbed or if such animals are fasting. Ruminants are not known to become deficient because of the vitamin supplied by the bacteria of the rumen. Thus



vitamin B_{12}

meats and especially liver are the only important dietary sources. Human requirements are estimated to be only in the neighborhood of a microgram daily.

Although the function of this factor has been exceptionally difficult to outline, vitamin B_{12} is believed to participate in the formation of methyl groups. Apparently it is not essential to methyl transfer. Whether methyl synthesis is concerned or not, the vitamin is required for the production of red blood cells.

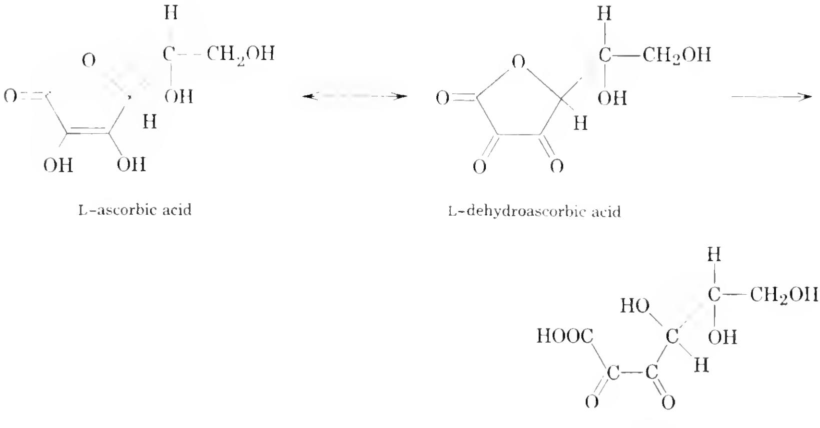
Pernicious anemia is not strictly a dietary disease, but in human beings and animals eating normally is due to improper absorption of vitamin B_{12}. This rather common and serious abnormality thus is a deficiency state but occurs only when the gastric juice is devoid of a second factor. The missing component normally forms a complex with the vitamin, permitting its absorption through the intestine. Many isolation studies have failed so far in isolating and characterizing a single material serving in the absorption of B_{12}. Apparently the factor is present in very small amounts and may actually be more than one substance.

Injection of the vitamin by-passes the problem of intestinal absorption and cures the deficiency symptoms. Anemia is the most common symptom, though others known either clinically or experimentally include mental disorders, reproductive failure, and necrosis of the liver, brain, and spinal cord.

ASCORBIC ACID

Chemistry

Also called vitamin C, this nutrient is a water-soluble vitamin but is not considered part of the B complex. Deficiencies of this substance have been reported for centuries as the disease scurvy. Famous accounts abound in literary works of all sorts and have encouraged attempts to isolate and characterize the vitamin. Now, of course, it is synthesized on such a scale that it sells for $13/kg. and is used in large quantities to fortify canned foods.



L-ascorbic acid

L-dehydroascorbic acid

L-diketogulonic acid

Vitamin C is a relatively strong reducing agent and is converted to dehydroascorbic acid. This product is as effective physiologically as ascorbic acid, probably because the reaction is readily reversible. The second reaction is not physiologically reversible. A postulated mechanism for the synthesis of ascorbic acid in plants has been presented on page 219. The vitamin is metabolized in animals, and parts of the molecule are eliminated as carbon dioxide, oxalic acid, and other products by an unknown mechanism.

Function

Much work has failed to clarify the physiological function of vitamin C. It has been suggested that this vitamin participates in the metabolism of tyrosine, in providing a suitable supply of Fe^{++} from the iron storage system, in the general maintenance of the necessary cellular oxidation-reduction potential, in certain terminal oxidations in plants, or in the synthesis of collagen or hyaluronic acid. In no case is the evidence clear cut.

Requirements

Apparently only the primates and guinea pigs need a dietary supply of ascorbic acid. Other species, including domestic animals and fowl, synthesize their own. Human daily requirements have been fixed at about 75 mg. for adults and from 30 mg. upward for children. Pregnant and lactating women should receive 100 and 150 mg., respectively. English workers, however, find that 10 mg. daily prevents all detectable symptoms of deficiency in adults and believe that 30 mg. provides an adequate margin of safety. There is no reason to challenge this lower value, nor is there any danger from a large quantity since massive doses are not toxic.

Ascorbic acid is widely distributed in fresh foods of all kinds, especially fruits and vegetables. The best of the common sources include strawberries, oranges, lemons, grapefruit, Brussel sprouts, cabbage, and spinach. Unpasteurized milk and fresh meat are relatively good sources.

Since this vitamin is a good reducing agent, it is lost under oxidizing conditions like aeration and heating in the presence of oxygen in neutral or alkaline solution. Thus many cooked or canned foods contain little ascorbic acid. Others, like canned tomatoes, are quite adequate when properly processed to limit the presence of air and metallic catalysts from the processing machinery. Certain enzymes also catalyze the oxidation of ascorbic acid and should be destroyed by blanching.

Pasteurized milk has lost much of its vitamin C, and the remainder is destroyed on standing. Hence the usual milk supplies are poor sources. Human milk contains three to four times as much ascorbic acid as cow's milk even when fresh from the cow and is obviously a much better source for infants. Synthetic vitamin C is now widely used in enriching canned foods, particularly fruits and juices. This procedure is especially important in baby foods since bottle-fed children receive so little ascorbic acid from milk.

Deficiency

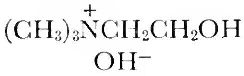
Lack of sufficient vitamin C causes scurvy, which is characterized by anemia, sore gums, loosening or loss of teeth, damage to capillaries with subcutaneous breakage, edema, and joint pains. In children these symptoms are accompanied by poor teeth and skeletal development with characteristic bone changes. Wound healing is retarded, and some investigators report an increased susceptibility to bacterial invasion. Except for the anemia, these symptoms are attributed to impaired formation of collagen and hyaluronic acid. These materials are known to be important in the maintenance of teeth and the skeleton and in the control of the permeability of tissues.

Scurvy has become rather rare over most of the world with cases seen only among persons with strong food prejudices, some remote tribes, and infants fed bottled milk and without fruits and vegetables. Formerly the disease was very common, especially among soldiers, sailors, and travelers of all kinds. In 1780 there were 1,457 men hospitalized with severe scurvy in a single British naval hospital. Daily rations of lemon juice throughout the navy cut this rate to a case each year in 1806 and 1807. Even though almost miraculous cures date back to at least 1535 during Canadian explorations, outbreaks of scurvy occurred through World War I in hospitals, asylums, and prisons. The toll of human lives taken by scurvy cannot be guessed at but is certainly huge. For example, Admiral Richard Hawkins stated in 1593 that 10,000 English seamen had died of scurvy within his own experience. During the voyage of Vasco da Gama around the Cape of Good Hope in 1498, 100 men died of his crew of 160.

OTHER WATER-SOLUBLE FACTORS

Choline

Although not a typical vitamin, this substance is usually classified with them for purposes of discussion. As might be guessed from the structure, this substance plays a role in the metabolism of methyl



$$(CH_3)_3\overset{+}{N}CH_2CH_2OH \quad OH^-$$

choline

groups (see pages 466 to 469). Choline occurs as a component of the lecithins. It is also important in the functioning of the parasympathetic nervous system, which depends in part on the presence of acetylcholine for the transmission of nerve impulses. When the nerves of this system are stimulated, they catalyze the formation of acetylcholine from choline and acetylcoenzyme A, using the enzyme choline acetylase. After the stimulation has occurred, acetylcholine might collect at the synapses or connectors between cells and cause continuous stimulation. This situation is avoided by the action of acetylcholine esterase, which yields acetate and choline, thus terminating the stimulation. Acetylcholine aids in controlling the heartbeat, for example.

Requirements for choline depend upon other dietary factors. High-fat diets impose a greater need for choline than high-protein diets. The latter provide methionine, which is also effective in methylations and can replace much of the choline. Betaine can replace choline completely, probably by conversion to it, and dimethylaminoethanol replaces choline in the chick but not the rat.

$(CH_3)_3\overset{+}{N}CH_2COO^-$
betaine

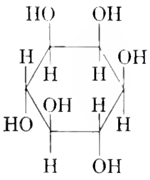
$(CH_3)_2NCH_2CH_2OH$
dimethylaminoethanol

When on normal diets, most animals are believed to require choline in much larger amounts than is the case with the ordinary vitamins. Chicks appear to need about 75 mg. daily, probably because the substance is a component of fats and acetylcholine in addition to its metabolic roles. Requirements have not been estimated for man. Choline is abundant in liver, heart, kidney, tongue, other meats, yeast, egg yolks, cereals, leafy vegetables, and a few vegetable oils such as soybean oil.

Deficiencies are not known in man except perhaps in pathologies where there is excessive wastage of body protein as in severe burns, wounds, or fevers. Some clinical use of choline has been made in such cases. Deficient rats develop fatty livers, kidney lesions, paralysis, anemia, hypertension, and edema, depending upon the severity and duration of the lack. Chicks show many of these same manifestations with another characteristic, one called perosis or slipped tendon.

Inositol

Hexahydroxycyclohexane occurs in nine stereoisomeric forms. Several of these occur naturally, and one called *meso*-inositol is widely distributed and of nutritional importance. This factor occurs in certain



meso-inositol

phospholipides best known from brain tissue and as phytic acid, the hexaphosphoric acid ester which occurs as the calcium-magnesium salt phytin in plants. Inositol is plentiful in heart muscle and in skeletal muscle of sharks, where it may serve as an energy reserve similar to glycogen in other species. It has been reported that inositol is a prosthetic group of α-amylase, important in carbohydrate metabolism. Roles have also been suggested for it in the metabolism of phenolic compounds and ketones.

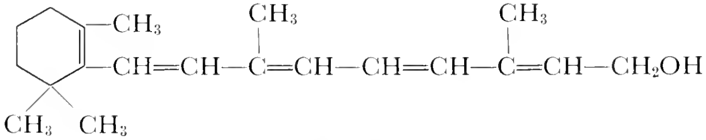
Inositol is apparently required by chicks, turkeys, pigs, and a variety of other animals. Daily allowances have not been established. Deficiency leads to loss of hair and under certain dietary conditions to the accumulation of lipides in the liver. Meat, bran, yeast, and grains are among the foods high in this factor.

VITAMIN A

This vitamin is a member of the group of food factors characterized by their solubilities in fat solvents. The other important members are vitamins D, E, and K. Certain fatty acids must also be supplied in the diets of various animals and are discussed on pages 460 to 462.

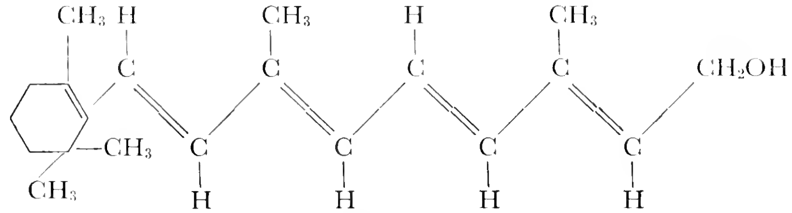
Chemistry

Vitamin A is sometimes designated vitamin A_1 to distinguish it from two less common forms, A_2 and A_3, isolated from fresh-water fish and lard, respectively. All are isoprenoids in that they can be represented as consisting of isoprene units. Vitamin A_1 is the most abundant, occurring in vertebrates except in most fresh-water fish and the gill-breathing forms of amphibians. Its structure is as follows:

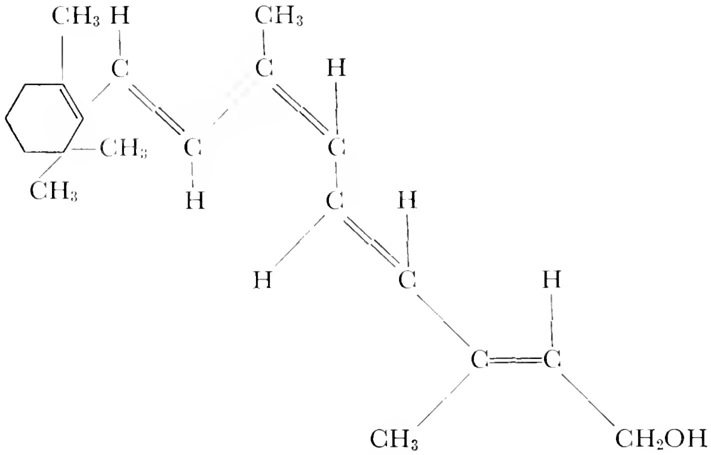


vitamin A_1

Vitamin A_2 differs in having another double bond in the upper left position of the ring structure written above. A_3 has been isolated only recently and its structure is not completely established. With several double bonds along the chain, a series of isomers of the *cis-trans* type is possible. Two of these are known to be important. *Trans*-vitamin A_1 has the *trans* configuration at all its double bonds, and the other type



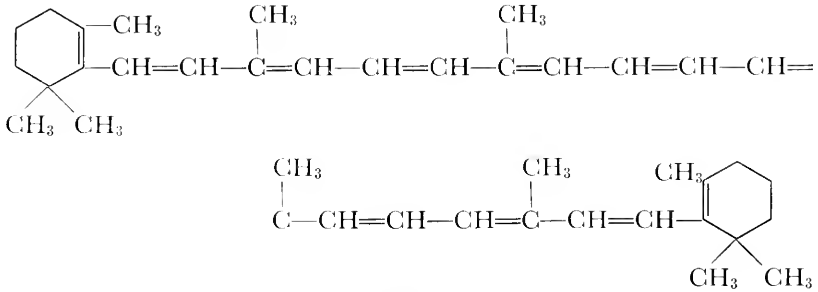
trans-vitamin A$_1$



Δ^3-*cis*-vitamin A$_1$

has one *cis* double bond, probably the third. Although unknown at present, others of the possible isomers may exist in nature.

Although plants do not contain vitamin A, herbivorous animals must either depend on plants for a substitute or synthesize their own vitamin. Actually all plant eaters probably follow a middle course and convert certain plant pigments into vitamin A. The most important pigment in this process, β-carotene, is already closely related



β-carotene

to the vitamin. Since both halves of β-carotene are alike and match vitamin A_1, the former should be readily converted to the latter by breaking the middle double bond and forming primary alcohol groups. The actual biological yield is still in doubt, but the β-carotene ingested is converted at least in part to vitamin A_1.

Obviously β-carotene might exist in a number of isomeric forms, and some of these have been studied. Moreover, similar compounds not isomeric with β-carotene also serve as pro-vitamin A substances. Some of these correspond to vitamin A through half the molecule while the other half differs. At least eight such biologically active carotenoids supplement the β-carotene to some extent. Most are of quite limited occurrence.

Function

A severe deficiency of vitamin A leads to a wide variety of symptoms. Hence this factor must have general metabolic effects, but none have been discovered in spite of a great deal of effort. The overall features of one specialized role, that involving vision, have been worked out.

Eyes of higher animals contain light-sensitive elements in the retinas especially adapted for seeing at low levels of illumination. These rods in turn contain a red photosensitive pigment called rhodopsin, a complex protein split by light. The known transformations are summarized in Figure 14–3. The intermediate designated retinene differs from vitamin A only in oxidation of the primary alcohol group to an aldehyde, and opsin (or scotopsin) is the name applied to the protein moiety.

Interconversion of the forms of vitamin A probably occurs in the livers of mammals and depends on the blood for transfer to and from the eye. Oxidation and reduction require an enzyme which is similar or identical to alcohol dehydrogenase. A direct interconversion of the two retinenes may occur. The combination of opsin and *cis*-retinene

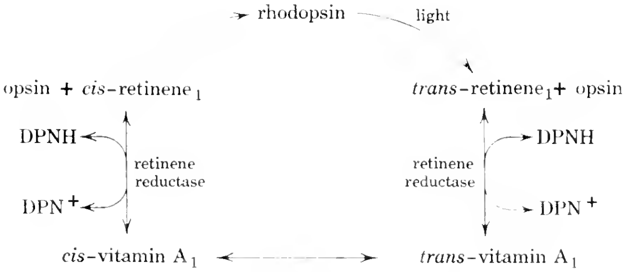


FIGURE 14-3. The visual cycle.

is endergonic and occurs without an energy-coupling mechanism. Decomposition of rhodopsin requires energy and is driven by absorption of light with the formation of certain poorly characterized intermediates.

The mechanism of nerve stimulation is still in doubt. One preliminary theory suggests that *cis*-retinene combines with and blocks —SH groups of the opsin. When these groups are freed during the photochemical reaction, it is postulated that they trigger nerve impulses. Whatever the mechanism, the process is remarkably sensitive and efficient. A single quantum of light dissociates a molecule of retinene from rhodopsin, and human beings can detect light in an amount just sufficient to split one molecule of rhodopsin in each of only 15 to 20 per cent of the rods present in the eye. Apparently there is a large magnifying effect between the photochemical event and the nerve impulse.

Vision based on the rods is without the sensation of color which is experienced only when a higher threshold is exceeded and the cones come into play. These sensitive elements are believed to contain pigments of three different colors responding to light of different colors. One has been isolated from chicken retinas and shown to be a protein complex with retinene$_1$ based on a protein different from that of rhodopsin. The change in protein alters the absorption spectrum of the complex and yields a pigment of different color. Perhaps three such complexes are sufficient to produce the nerve stimulations we know as color.

Requirements

For nutritional purposes vitamin A is still expressed in international units equal in effect to 0.6 μg. of β-carotene. Diets should contain one or the other or a combination equivalent to the recommended daily allowances. Vitamin A is stored extensively in the liver, from which it is withdrawn at need. However, the presence of a large reserve complicates the estimation of the supply regularly required.

Various studies suggest that 1,300 units daily may be adequate for human beings, but it is customary to increase the figure to 3,000 to 5,000 for adults and 8,000 units during lactation in order to provide a safety factor. Allowances for beef cattle range from 25 to 55 mg. of carotene, depending on body weight, with 2 to 40 mg. for swine. Poultry feeds should contain 2,000 to 3,300 units of vitamin A, depending on age, type, and physiological activity, with 4,000 units for breeding turkeys.

Carrots, sweet potatoes, outer leaves of lettuce and cabbage, apricots, oranges, peaches, tomatoes, alfalfa, and pumpkins are good sources of carotene. Yellow corn is the only cereal containing significant amounts. Eggs, liver and fish-liver oils are high in vitamin A, while milk and butter are rich in both carotene and vitamin A. It is now customary to fortify many foods with one or the other factor, especially oleomargarine and shortenings. Vitamin A is relatively unstable in air unless protected by natural or added antioxidants, including vitamin E.

Deficiency

Severe deficiency symptoms occur in adult animals only after prolonged deprivation. Human beings previously on good diets show little effect after 1 to 2 years without carotene or vitamin A because of the supply stored in the liver. Shortages in the diets of very young animals produce rather rapid and severe symptoms. Starting first with night blindness, other malfunctions appear, including poor growth, kidney stones, reproductive failures, degeneration of nerve tissues, abnormal bone growth, degeneration of epithelial tissues followed by secondary infections, and characteristic drying and destruction of the cornea, leading to blindness. Rather complete recoveries are common if treatment is administered in time. Night blindness always seems to be the earliest change, potentially a serious matter where the victim drives a car, especially since he is never conscious of his disability.

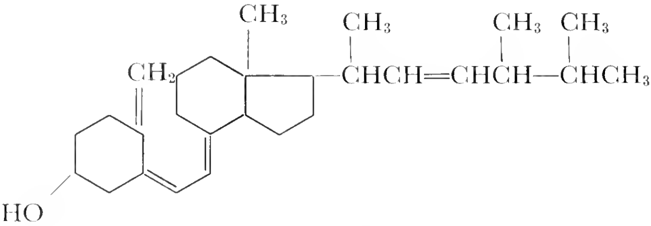
Deficiencies are probably rare in people of western countries at present but were encountered in significant numbers until the end of World War I. Danish children suffered especially because of export of the dairy products to Germany. Lack of vitamin A is still the chief cause of blindness in children in India, China, and southeast Asia, where the cases are numerous and two-thirds result in blindness.

Repeated great overdosage with vitamin A can cause fragility of the bones or swelling and soreness in rats. Doses of 500,000 units daily for extended periods to infants have produced moderate symptoms. Illness and even death have been reported in Arctic exploring parties as a result of the consumption of polar bear liver, an extremely concentrated source of Vitamin A.

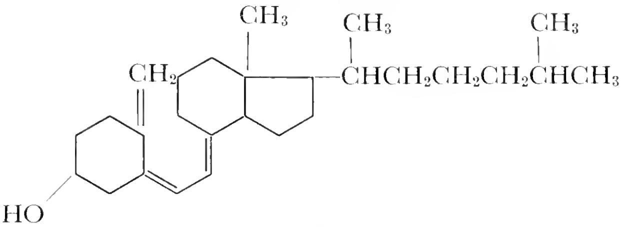
VITAMIN D

Chemistry

A number of related compounds possess vitamin D activity in the prevention and cure of rickets. Of these only two are of much practical importance. The plant sterol ergosterol (page 94) is changed on irradiation into vitamin D_2, and an animal counterpart, 7-dehydrocholesterol derived from cholesterol (page 93), is converted to vitamin D_3. These substances differ only in their side chains and in common with the other active compounds have the same structures otherwise. Names approved internationally for these substances but



vitamin D_2 (calciferol)



vitamin D_3

not yet in common usage are ergocalciferol and cholecalciferol. The latter compound, vitamin D_3, is the form stored by fish in their livers and is natural to mammals exposed to sunlight. Both compounds are available commercially in large quantities at 2.5¢/million units.

Function

For most animals D_2 and D_3 can be used interchangeably, but birds cannot utilize calciferol and must have vitamin D_3. The reasons for this difference are not known.

Vitamin D plays a role in the calcification of bones and teeth. This hardening process may be retarded by deficiencies of calcium, phosphorus, or the vitamin. Unusual proportions of the two elements in the diet may also be detrimental. The mechanism by which vitamin D promotes normal deposition of calcium phosphate is unknown. It has been suggested that the vitamin participates in the intestinal absorption of the minerals. Very recently it has been observed that D_2 reduces the oxidation of citrate and isocitrate; as a result of this function, the vitamin may control the concentration of these two metabolites. Since these compounds are important complexing agents for cations, this effect may control in turn the transport of calcium ions to the bones. A great deal of work will be necessary to establish this or any other theory on a sound foundation.

Requirements

Vitamin D allowances are still expressed in international units or in chick units, each equal to 0.025 μg. of pure vitamin D_2 or D_3.

It is recommended that infants, children, young adults to the age of 20, and pregnant or lactating women receive 400 units daily. In cases of active rickets prescribed daily doses may range from 500 to 1,500 units. Growing chicks and laying or breeding chickens should receive 180 and 450 chick units of vitamin D_3/lb. of feed. The allowance for young and breeding turkeys is 800 chick units/lb. of feed. Swine require 135 to 625 units, depending on size, and cattle about 300 units/100 lb. of body weight.

The best natural sources of vitamin D are the oils of fish livers. Egg yolks are fairly good, but milk is poor. Hence it is becoming more common in western countries to enrich milk and margarine. Individuals with a reasonable proportion of their skin area regularly exposed to sunlight require little or no supplementary vitamin D. That formed in the skin as a result of the irradiation can be quite ample, as shown by examination of people living outdoor lives in warm climates.

Continued excessive doses of vitamin D are hazardous, producing abnormalities of bones and teeth as well as kidney stones. This rather rare result has been obtained experimentally and observed on occasion in children inadvertently given too much of this material.

Deficiency

The deficiency disease rickets is primarily a disease of growing bone with the abnormality occurring at the final stage of mineralization. As a result bones may become flexible and misshapen, leading to enlarged skulls and joints, knock-knees or bowlegs, beaded ribs, and protruding chest. Muscular impairment, poor tooth development, and pot bellies are other standard results.

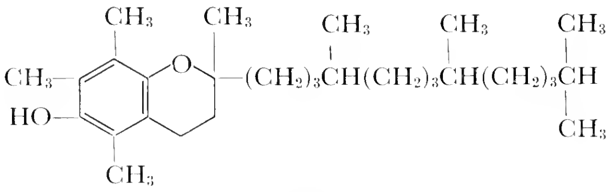
These symptoms are characteristic of rickets in young and growing animals and may be observed in pregnant or lactating females. The disease is still fairly prevalent among women in many parts of India, China, Egypt, and other countries with low living standards and a custom of keeping women indoors or completely clothed, thereby preventing vitamin production by irradiation of the skin. Within the generation it has been reported that in northern China 2 to 5 per cent of pregnant women die in labor from the adult form of rickets.

Among children severe rickets is still common in many areas, and mild rickets was demonstrated in almost half of the 700 children dying of all causes in Johns Hopkins Hospital in 1940 to 1942. In England as late as 1920 there were 1,760 children in elementary school with rickets of crippling degree. Even in 1944 there were estimated to be 24,000 cases of mild rickets in Great Britain in children under a year old. Fortunately these symptoms had largely disappeared when the children became older. Although still a problem even in the West, this disease is not so common as it once was. In 1868 one-third of all London children had severe rickets. Most of the others probably had mild cases.

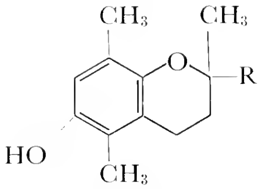
Rickets is most prevalent where climate or custom prevents exposure to the sun. Crowded urban and heavily industrialized northern areas in the winter months tend to produce the greatest need for dietary vitamin D. Except in fish this vitamin is not stored so extensively as is vitamin A. Apparently at least weekly supplies of D are needed.

VITAMIN E

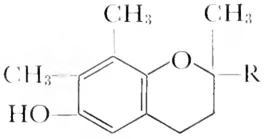
At least 40 natural and synthetic compounds are known to have some activity as replacements for α-tocopherol, the most abundant and one of the most effective forms of vitamin E. Three other tocopherols have been isolated, all with high vitamin activities.



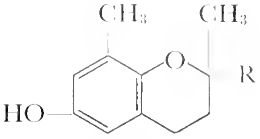
α-tocopherol



β-tocopherol



γ-tocopherol



δ-tocopherol

R stands for the side chain of α-tocopherol. These four forms of vitamin E thus differ only in the number of methyl groups on the aromatic ring.

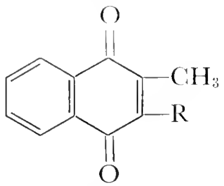
In vitro the tocopherols are good antioxidants and retard development of rancidity in fats by preventing the aerobic autoxidation of the carbon chains of unsaturated fatty acids. Vitamin concentrates are used to some extent in foods to prevent the development of the flavors and odors characteristic of oxidative rancidity. Synthetic antioxidants administered to deficient animals cure most of their deficiency symptoms, so vitamin E is thought to function in animals primarily but perhaps not exclusively as an antioxidant.

Vitamin E is known to be required by all the laboratory and domestic animals studied. Presumably it is required by man as well, but there is no direct evidence. Reliable estimates of daily allowances are not yet available and perhaps not of practical importance, since the amounts in ordinary foods and feeds seem quite adequate. Wheat-germ oil, cottonseed oil, and soybean oil are rich sources with small amounts in meat, milk, eggs, leafy plants, and some fruits.

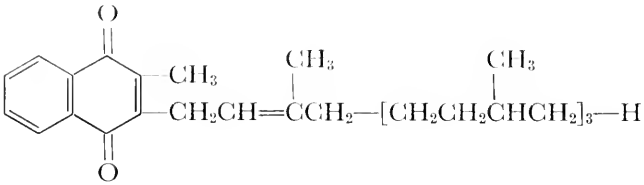
Deficiencies in human beings are not definitely known and probably are very rare. Likewise they are probably rare in animals outside the laboratory. Herbivores may be slightly more prone to deficiency symptoms, for they are more sensitive than are other animals to special diets in the laboratory. Deficiency symptoms under experimental conditions are reproductive failures, including poor hatchability of eggs, kidney lesions, and muscular distrophy. Addition of vitamin E to the diet reverses early stages of these changes but has little effect on the miscarriages, abortions, sterility, and muscle degenerations commonly encountered in animals on natural diets. Presumably these same symptoms also arise from other causes than deficiency of tocopherols.

VITAMIN K

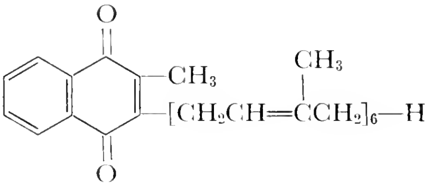
Two forms of this vitamin have been isolated and many more active substances have been synthesized. Most of these are naphthoquinones with the general formula shown or may be converted to such quinones in the body. The two natural vitamins have been iso-



lated from alfalfa and from fish meal, and their respective structures are as follows:

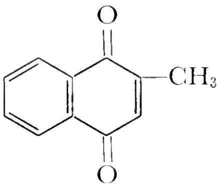


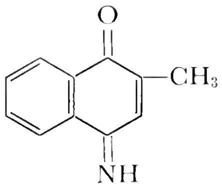
vitamin K_1



vitamin K_2

Two other forms are widely used clinically under various trade names. Both are extremely effective. When a water-soluble form is required





in cases of difficulties in the absorption of lipides, the hydrochloride of the last compound is used.

Vitamin K has a single known function. It is required for the formation of one of the components of the prothrombin system essential for the clotting of blood when the circulatory system is damaged. The mechanism by which this function is performed is quite unknown.

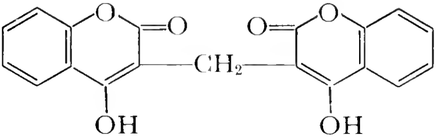
This factor is rather widely distributed and is abundant in green leaves. It is extensively synthesized by the intestinal flora of the animals concerned. All higher animals probably require this vitamin, but under normal conditions intestinal synthesis is adequate even if the diet is not. Daily allowances cannot be recommended at present.

Deficiencies can be produced on laboratory diets which include sulfa drugs and the like to prevent vitamin synthesis in the intestine. Spontaneous subcutaneous hemorrhages are observed and prolonged or fatal bleeding results from minor injuries.

In man the pathologies that interfere with the intestinal absorption of lipides lead to vitamin K deficiency. These include obstruction of the bile duct or intestine, failure of the pancreas, and the disease sprue. Surgical remedies, where possible for these conditions, require prior use of a suitable drug tc prevent death from hemorrhages during the operations.

Both internal and external bleeding is observed in about 10 per cent of newborn children and persists for 2 to 6 days without vitamin treatment. This sometimes serious problem is due to lack of a suitable intestinal flora and consequent vitamin K deficiency. Reduced bleeding of the mother during delivery is reported when K is given before or at the onset of labor. Such treatment also supplies enough of the vitamin to the infant to allow time for the formation of a normal flora.

Cattle on sweet-clover pastures are subject to sweet-clover disease, a typical vitamin K deficiency due to the occurrence of a natural antagonist for the vitamin in the plant. This antivitamin is used medically in the prevention of blood clots where there is danger of thrombosis, especially during certain surgical procedures. The dosage is rather critical since too much induces hemorrhaging. The antivitamin is called dicoumarol.



dicoumarol

VITAMIN ASSAYS

Many kinds of methods are used in the estimation of the various vitamins found in different foods and feeds. The development of accurate determinations is not as simple as might at first appear. The great complexity of the materials to be examined makes likely the occurrence of substances interfering with the measurements. Many instances of serious error of this and other types might be cited, and others can be reasonably expected in the future. Thus some sort of standards to be met by the analytical methods employed will at least aid in reducing the size and frequency of errors.

Criteria for Useful Assays

Whatever the method, it should satisfy the following conditions if at all possible.

1. Values obtained at various levels of the factor in the assay should agree over at least a four-fold range of concentration. In other words, quadrupling the amount of the factor should produce a proportional increase in the property measured.
2. Consistent values should be obtained on replication at different times.
3. The assay must agree with other acceptable methods of analysis within reasonable limits of experimental error.
4. The method should be specific for the factor under test. Test systems or organisms should be supplied with sufficient materials of all other kinds in order that the one in question be the only variable.
5. Recovery values at various concentration levels should agree with known amounts added. Recovery values are obtained by mixing known amounts of the factor with standard quantities of the unknown source. This procedure checks for interference from the unknown source and should give additive values.
6. There should be no drift in results; that is, there should be no trend either high or low in the analysis of standard samples.

Satisfaction of these criteria indicates a useful method for assay of vitamins or almost any other physiological factor. Since the number of methods is becoming enormous, procedures cannot be discussed in detail. For the present, the assays are grouped into the following four categories.

Biological Methods

As the name implies, biological methods depend on the use of experimental animals to determine the presence or absence of a specific vitamin in a food mixture. Animals most commonly chosen for vitamin assays are rats, mice, pigeons, and chickens. Guinea pigs are employed almost exclusively for biological estimations of ascorbic acid. Hamsters have been used for certain types of vitamin assays, and higher animals, such as the dog, have been utilized to a limited extent.

In general the principles involved in all biological assays are similar. Control groups of animals are fed a so-called chemically purified ration consisting of all known nutrients (protein, fat, carbohydrate, mineral salts, and vitamins) necessary for normal health and growth. This control ration contains optimal amounts of all vitamins, including the vitamin to be tested.

Similar groups of experimental animals receive the same ration as that fed to the control animals, except that the vitamin to be measured is omitted. As a result these latter groups develop typical symptoms of vitamin deficiency which can be prevented or cured by addition of the food material containing the vitamin to be assayed. Biological assays are of two general types: (1) prophylactic, and (2) curative.

Prophylactic tests are made by adding small graduated amounts of the food to be tested to the vitamin-deficient ration at the beginning of the feeding tests. In this way it is possible to find the least amount of food necessary to prevent vitamin-deficiency symptoms and to produce a rate of growth comparable to that obtained with the complete control rations containing known amounts of the vitamin. When the curative methed is used, several groups of animals receive the vitamin-deficient ration until they begin to lose weight and develop symptoms of vitamin deficiency. At this point control groups receive a sufficient amount of the pure vitamin to cure the deficiency symptoms and cause the animals to grow at a required rate. Comparable experimental groups of vitamin-deficient animals receive graduated amounts of the food to be tested until an amount is found which contains sufficient vitamin to bring about growth responses comparable to those obtained with the control animals.

Reference standards in the form of chemically pure vitamins are used for control studies whenever possible. Crystalline thiamine hydrochloride, riboflavin, and L-ascorbic acid are examples of pure reference standards used in assaying foods for vitamins B_1, B_2, and C, respectively. Crystalline β-carotene was the international standard for vitamin A assays, but it has been superseded by crystalline vitamin A acetate.

Vitamin D is determined biologically by producing vitamin D deficiency (rickets) in rats and finding the minimum amount of food required to initiate recalcification in the bones. This method involves sacrificing the animals in order that the bones can be examined microscopically. When chickens are used for vitamin D assays, the prophylactic method is followed, and the ash content of the bones is considered the criterion of vitamin D potency. Rations of a special type, containing abnormal calcium-phosphorus ratios, are used for the production of experimental rickets. In some of these biological methods, satisfaction of the first criterion of page 362 is impossible.

Chemical Methods

In certain cases satisfactory colorimetric or fluorimetric methods have been standardized against biological assays and are now widely used because they are much quicker and less expensive than the latter.

Colorimetric methods depend on the formation of stable colored compounds or complexes that can be measured in a colorimeter. Vitamin A forms a blue complex with antimony trichloride in chloroform. When vitamin A is extracted from a saponified food material, it can be treated with the antimony trichloride reagent, and the color can be compared with that produced by known amounts of pure vitamin A, using the same reagent. The depth of color is proportional to the concentration of vitamin A.

Vitamin C can be titrated with a purple dye called 2,6-dichlorobenzenoneindophenol. The dye is a mild oxidizing agent which oxidizes L-ascorbic acid to dehydroascorbic acid. In the process the purple dye is reduced to a colorless compound. Thus the dye serves as its own end-point indicator. Pure crystalline ascorbic acid is used as a reference standard, and the vitamin content of the unknown food is determined by comparison with this standard.

Fluorimetric methods depend upon the presence of a fluorescent substance, one that absorbs light and re-emits light of a different wave length. When the incident light is employed as a beam, the fluorescent light is emitted in all directions and is readily detected with a photoelectric cell placed at right angles to the incident beam. Oxidations of thiamine and riboflavin with a mild agent yield products that fluoresce when irradiated with ultraviolet light. When pure vitamins are used as reference standards, the unknown quantities in foods can be estimated readily.

Physical Methods

Vitamins A and D have characteristic absorption spectra and are determined spectrophotometrically. The instrument used for this work is called a spectrophotometer. It measures the amount of light of any chosen wave length absorbed by a solution of the sample. The wave lengths absorbed depend upon the molecular structure, primarily on the number and arrangement of double bonds in the molecule. A range of wave lengths affected by the molecule is called an absorption band. In such an absorption region, the amount of light absorbed is directly related to the concentration and is used to estimate the latter. Most such measurements do not require much material, and what is used is readily recovered after the analysis. Spectrophotometric procedures are widely employed in determining a great variety of materials and are closely related in theory to the colorimetric methods of the previous section.

Microbiological Methods

Numerous microorganisms require vitamins for growth, particularly the members of the B group. Since different species and strains have different requirements, a form can be selected that requires the factor in question for normal growth. When all other necessary factors are supplied in suitable amounts, the organism still does not multiply until the vitamin to be assayed is added. Thereupon, the rate or the total amount of growth depends upon the amount of the vitamin added. Growth may be determined by microscopic counting, plate counts, turbidity measurements, nitrogen determination on the washed and collected cells, or in certain cases the titration of acids formed in the medium by the growing culture. Methods are calibrated with known amounts of pure vitamins.

Many types of organisms are used in vitamin assays, and other classes of compounds are determined in a similar way. Bacteria, fungi, and yeasts are used to detect and measure vitamins, amino acids, cations, and metabolic compounds of various kinds.

To cite a typical example, the acid-producing bacterium *Lactobacillus casei* will not grow normally if the nutrient medium is lacking in folic acid. Control tubes containing media deficient in folic acid are supplemented with graduated amounts of pure folic acid, inoculated with a pure culture of *L. casei,* and incubated for a specified period. The acid produced in each tube is measured by titration with a standard alkali solution. Similar tubes containing graduated amounts of the food to be tested are treated in a similar manner, and the amount of acid produced is titrated. By comparing the acid production in the experimental tubes with the acid production in the control tubes containing pure folic acid, the amount of folic acid in the food sample under examination can be estimated with speed and accuracy.

VITAMINS AND NUTRITION

It appears that the point of diminishing returns is rapidly being reached in the use of the classical approaches to the discovery of new vitamins. The recognition of diseases as dietary and the subsequent identification of missing factors have been the primary driving forces in the discovery of the vitamins now recognized. However, it is unlikely that observations in human medicine will be of much further help. Perhaps abnormalities in other animals will lead to new discoveries as in the case of vitamin K, first shown to be lacking in certain artificial chick diets. Since microorganisms are more diverse in their nutritional requirements and metabolic capabilites than are higher plants and animals, the search for nutritional factors in this area is more likely to turn up vitamins having general application.

No higher animal has been grown on a diet of exclusively synthetic materials. Therefore, additional vitamins are to be expected. Yet an exhaustive analysis of the known and unknown compounds in the diets of higher animals presents staggering problems. A quicker approach would appear to be the search for factors required for the maintenance and growth of animal tissues in laboratory cultures. Experiments should be easier, cheaper, and quicker and the requirements simpler than for intact animals. Eventually a combination of the factors required for different isolated tissues should support the whole animal.

Much remains to be done in the matter of optimal nutrition. Our present safety factors in vitamin allowances may be either inadequate or excessive and reduce growth, bodily function, or longevity. This problem is complicated by the effect changes in other dietary components are known to have on minimal vitamin requirements. Perhaps the optimal values also fluctuate with the overall diet. The importance of such work is emphasized by reports that longevity is improved in rats fed the minimal allowances rather than larger quantities of vitamins.

This problem is one aspect of the balanced diet. It is now widely accepted that diets containing all the required factors but low in total quantity are superior to diets adequate in quantity but quite deficient in any single essential substance. Pronounced pathological disturbances invariably appear in the latter situation. The former normally leads to stabilized body weight at a lower level but without impairment of function unless the diet is drastically meager.

Present opinion in America is swinging to the position that the major cause of malnutrition in this country is overeating. Anyone weighing 20 per cent over the accepted standard for his height and skeletal structure is pathologically overweight and can anticipate a diminished life expectancy. Disorders of the circulatory system are the most common result. It is estimated that the nutrition of at least

5,000,000 Americans is poor to the extent of 20 per cent excessive weight. Instances of pathological overweight from other causes certainly occur but are rare by comparison. In the usual cases people just eat too much. Malnutrition of this kind is probably social and psychological to a great extent.

In economically poor countries, malnutrition takes the form of starvation and, when less acute, of the vitamin-deficiency diseases discussed throughout this chapter. Another very widespread dietary disease called kwashiorkor is basically due to lack of protein complicated with vitamin deficiencies. This disease, which may now be the most widespread of the serious dietary disorders, kills large numbers of people annually. It is quite common among the poor classes of Latin America, Africa, India, and other areas depending on grains without adequate meat or fish.

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Mineral metabolism

15

This title is broadly interpreted to cover all the biochemical relationships of the metallic elements but is specifically restricted to animals in this chapter. In addition, certain non-metals are essential to animals and except for hydrogen, oxygen, carbon, and nitrogen these are discussed here too. The four elements excepted are separately covered in the chapters on the metabolism of carbohydrates, lipides, and proteins. These same chapters deal with the general metabolism of sulfur except for certain special problems of sulfate taken up below.

NUTRITION

The various ions believed to be essential for a variety of the higher members of the animal kingdom are taken up in alphabetical order. This sequence is chosen because one cannot assign degrees of requirement to the elements concerned. Obviously the concept of essentiality means that complete deprivation leads to death and partial deficiencies to physiological impairment. Experiment supports this basic concept.

Naturally the information available differs in quantity and diversity for the different elements. This situation has arisen from the personal interests and prejudices of investigators, accidental factors, availability of basic biological and chemical knowledge, existence of suitable apparatus and procedural techniques, lack of ideas in some cases, and differences in innate investigational difficulties. One cannot conclude that any one of the elements listed is more important than another because more is known about it. Nor is it safe to say that any element is non-essential, for it is philosophically impossible to prove that an element is not required.

On the contrary, as methods are devised and improved new members appear on the list of required elements. Therefore, the group classified as incidental elements includes those found in animals, though for several of these there is no known role or requirement in animals. A few have the special functions mentioned in particular species.

Where the available information warrants, the special biological roles, dietary requirements, deficiency states, toxicities, excretions, and physiological interactions are discussed for each ion listed. Certain more generalized roles are considered in four sections at the end of the chapter.

Calcium

This most abundant inorganic element of the higher animals is located predominantly in the skeletal tissues, where it plays a primary role. Outside the bones and teeth calcium is distributed in small amounts throughout all the tissues where it has special roles in controlling nerve and muscle action, blood clotting, and cell permeability. The intercellular fluid averages about 20 mg. of Ca^{++} per 100 g. of tissue. Of this quantity some is in free form and some is complexed with proteins.

Part of the soluble calcium participates in the transmission of nerve impulses. The overall process was mentioned on page 306 and depends in part on the formation of acetylcholine from choline and acetyl CoA in the presence of choline acetylase. The catalytic activity of this enzyme requires both calcium and potassium ions. Thus without Ca^{++} acetylcholine is not formed and transmission of impulses along the nerves does not occur.

Stimulation of a muscle fiber by a nerve impulse leads to contraction of the fiber when the biochemical environment is suitable (see page 303). Although the detailed mechanism is in doubt, Na^+, K^+, and Ca^{++} are required. Sodium apparently is necessary for the contractility of animal muscle in contrast to potassium, which has a paralyzing effect. Since K^+ is always present, the concentration might often rise to levels interfering with muscular function. However, Ca^{++} antagonizes this property of K^+ and maintains a state of balance. Hence, though indirect, this role of calcium is of prime importance in muscle. More directly, Ca^{++} affects the activities of two enzymes found in muscle and thought to play roles in making the chemical energy of ATP available in the form of muscular work. Both enzymes catalyze:

$$\mathrm{ATP} + \mathrm{H_2O} \rightarrow \mathrm{ADP} + \mathrm{HOPO_3^{=}} + \text{energy}$$

one being activated by calcium, the other inhibited by this ion but activated by magnesium.

Since higher animals possess endoskeletons, their complex circulatory systems are relatively exposed to mechanical damage. The evolutionary development preventing loss of the circulatory fluid is termed blood clotting. This complex process (see pages 296 and 297) requires Ca^{++} in at least one step. Thrombin thus formed catalyzes the conversion of fibrinogen to fibrin, which polymerizes to form the clot. Therefore, otherwise trivial injuries become serious when the Ca^{++} concentration of the blood is low. For experimental uses, blood clotting is readily prevented by addition of any material precipitating or complexing with the calcium ions.

$$\text{Prothrombin} \xrightarrow[\text{thromboplastin}]{Ca^{++}} \text{thrombin}$$

The permeability of cells is affected by the presence of ions, particularly by divalent cations and most especially by Ca^{++}, which precipitates or forms complexes with so many anions. Association of the Ca^{++} with the cellular membranes controls the charge on the membrane, which in turn controls the diffusion of soluble electrolytes by way of electrostatic interactions. Even the penetration of non-electrolytes into cells is affected by the presence of Ca^{++}, which forms organic salts with the carboxyl groups of the membrane materials. These calcium salts are much more readily wet by lipides than are most polar compounds occurring biologically. Passage of relatively non-polar molecules into cells may be dependent upon this property of calcium compounds.

Since the proper functioning of these several physiological systems depends on the maintenance of a rather definite concentration of calcium ion, some control mechanism must be operating. Inasmuch as the concentrations in all other tissues depend on that in blood, regulation of the level in the blood suffices for the animal as a whole. The necessary control is exercised in some unknown way by a hormone secreted from the parathyroid system into the blood. Removal of these little glands located near the thyroid leads to reduction in the Ca^{++} level of the blood and increased excretion to the point of deficiency symptoms. It has been suggested that the hormone may function by controlling the permeability of kidney membranes to Ca^{++} and thus limiting the rate of excretion.

Whenever the diet is low in calcium, excretory losses from the blood are replaced with Ca^{++} drawn from the skeleton. Teeth probably do not serve as a useful reservoir in such cases. Conversely, when calcium is absorbed in large quantities, the excess is stored in the skeleton. Prolonged excess assimilation of calcium can lead to the abnormal deposition of insoluble salts, particularly as kidney stones.

The quantity of calcium needed by animals depends upon the species, rate of growth, physiological activity, and diet. The last is especially important for this ion, since calcium forms so many insoluble salts rendering it unavailable for intestinal assimilation. Thus phytic acid (page 351) can be sufficiently plentiful in plants to precipitate the calcium and reduce its absorption below the level needed. Abnormally large concentrations of phosphate have a similar effect, as does a deficiency of vitamin D.

Daily allowances with a safety margin have been set at 1.0 g. of Ca^{++} per day for adults. Pregnant and lactating women should receive, respectively, 1.5 and 2.0 g. per day. Children should receive from 1.0 to 1.4 g. daily, depending on age. Milk, legumes, eggs, and vegetables are the best sources.

Daily allowances for dairy cattle have been recommended as follows: 8 g. for animals weighing 100 lb., 14 g. at 400 lb., 15 g. at 600 to 800 lb., 12 g. at maturity (1,200 lb.), 22 g. during the latter part of pregnancy, and 1 g. per lb. of milk produced during lactation. It is suggested that daily rations for beef cattle should contain 20 g. of Ca^{++} at 400 lb. live weight and 15 g. at 1,000 lb. Bulls should receive 24 g. at 600 lb. and 18 g. at 1,800 lb. Wintering calves and yearlings are allotted 16 g. and animals in the feed lot 20 g.

Young pigs of 50 lb. should receive 7.4 g. of calcium daily. At 100 lb. the recommendation is 13.7 g., and at 200 lb. it is 17.9 g., which is suitable for larger hogs. Pregnant animals and young boars should be fed 16.4 g. daily, and lactating sows and breeding boars from 27 to 41 g. of calcium.

Starting chick rations should contain 1 per cent calcium, increased to 2.25 per cent for laying and breeding hens. The last groups may receive the element in the form of oyster shell fed "free choice." Tentative allowances for turkeys are 2 per cent of the ration for poults and 2.25 per cent for breeding birds.

Protein concentrates of animal origin, such as tankage and fish meal, are good sources of calcium. Legume hays are also good, but the grasses and seeds are rather low and should be supplemented. More detailed information on both requirements and sources is presented in tabular form in the Appendix.

Calcium deficiencies are primarily of three types. One type is characteristic of rickets, showing poor bone and tooth development and skeletal malformations. These symptoms are discussed somewhat more fully in connection with deficiency of vitamin D. The second type, failure of the blood-clotting mechanism, represents a symptom of severe, acute lack of calcium and probably does not occur except in laboratory studies. The third type is the nervous disorder, tetany, characterized by intermittent convulsive muscular contractions. Diets high in oxalic acid and similar Ca^{++}-removing agents are occasionally encountered in nature and produce tetany.

Many nutritionists feel that the diets of many Americans are marginally deficient in calcium. The increased consumption of milk, especially among children, has been the most effective means of combating this problem. A greater use of leafy vegetables has also been helpful.

Animal deficiencies have been encountered under agricultural conditions in cattle, sheep, hogs, and chickens. Poor skeletal development, reproductive disturbances, low milk production, and soft-shelled eggs are the most common symptoms. "Grass tetany" disease may be a Ca^{++} deficiency and is reported in cattle in localities of Holland, New Zealand, England, and Ireland. In the United States highly acid soils in parts of Florida, Louisiana, and scattered pasture lands in Ohio and along the Atlantic and Gulf coasts produce grasses that are too low in available Ca^{++} without liming to raise the pH. Occasional acute deficiencies can develop, usually in the form of tetany, when either animals or human beings eat large quantities of plants high in oxalic acid. Rhubarb is probably the commonest cause of this condition.

Abnormally heavy intakes of Ca^{++} can lead to physiological disturbances, primarily in the form of excessive calcification of joints and deposition of solids in soft tissues. The salts formed are the oxalate, phosphate, and carbonate. Kidney stones are perhaps the most frequent manifestation.

Calcium is excreted in feces as insoluble forms not absorbed from the diet. Moreover, animals on calcium-free rations excrete calcium which presumably must be transferred back through the intestinal wall. Soluble calcium is also lost steadily in the urine, the quantity depending on the state of the animal and the amount absorbed from the diet.

Chloride

Animals get their chloride largely in the form of the sodium salt. The two ions are closely associated in many of their roles. Chloride and sodium are particularly important in the maintenance of proper osmotic concentrations in fluids and cells. Furthermore, chloride is involved somewhat indirectly in the transport of carbon dioxide by the blood. The chloride ion is important as a major electrolyte in controlling the solubility of proteins, especially the globulins.

Chloride is found throughout the tissues of animals and is adsorbed to a considerable extent to proteins, altering their zeta potentials. About two-thirds of the anions of blood are Cl^- with a normal concentration in the serum near 0.36 per cent. The interstitial fluid is about the same. Red cells, on the other hand, range from 0.19 to 0.23 per cent Cl^-, and urine contains about 0.60 per cent Cl^- more or less. Blood levels depend to a small extent on the quantity ingested, the volume of liquid drunk, and the amount lost in urine and perspiration. The chloride in the erythrocytes varies during the ionic exchanges accompanying carbon dioxide transport by the cells. In urine the concentration of chloride varies much more and depends closely on the amount of Cl^- eaten and the volume of urine, thus ranging widely from time to time during the day.

Under normal conditions human beings and animals need dietary Cl^-, but the quantity essential is difficult to ascertain. On a diet not containing chloride, none is excreted in the urine after a few days. Thus the body effectively conserves its supply. Since plants tend to be somewhat low in both Na^+ and Cl^-, herbivores need supplementary salt. Animal tissues are high in salt by contrast, and the carnivores get plenty in their usual foods. Omnivores like man and swine ordinarily get enough Cl^- unless restricted to purely vegetable diets.

Deficiencies occur in cattle, sheep, and even deer unless salt is made available. The porcupine is legendary in his patient search for salt. Even horses may need salt in hot weather while working hard and sweating profusely and repeatedly. Human beings develop deficiencies under similar conditions. For example, miners working at elevated underground temperatures may lose salt at the rate of 2 g. per hour, which must be replaced if the loss is at all prolonged. Deficiencies may also occur after extended vomiting or diarrhea and are characteristic of Addison's disease, a kidney malfunction leading to rapid loss of both Na^+ and Cl^-. The symptoms of salt deficiency include abdominal cramps, weakness, rapid loss of weight, and cardiovascular disturbances. In severe cases cerebral edema causes mental confusion, and there is collapse of circulatory function and death.

An excess of salt raises the osmotic concentration of the extracellular fluid and withdraws water from the cells. If extensive, drastic changes occur leading to severe metabolic disturbances, hallucinations, and death. Animals normally do not eat enough salt to produce this condition except when drinking sea water in the absence of fresh water. Then, of course, the salts of the sea water merely dehydrate the cells more quickly and intensify thirst.

Of the ingested Cl^-, 90 per cent or more is excreted in the urine, about 5 per cent in perspiration except during heavy exercise in hot weather, and perhaps 1 per cent in feces. None of these figures can be considered fixed indexes because all are markedly affected by the rate of ingestion, physical activity, liquid consumption, and environmental temperature.

Cobalt

Since vitamin B_{12} (page 345) probably occurs in the cells of all animals and is essential for their normal metabolism, cobalt must be supplied at least in this form. Certain peptidases from mammalian tissues require metal activators, and Co^{++} is the most effective ion. Since other metals do work *in vitro*, it is difficult to know whether cobalt plays this role inside living cells or not. This same situation exists for a few other known enzymes, so it is possible that cobalt plays other required roles than that in vitamin B_{12}.

Daily allowances have not been set with any degree of confidence because of the exceedingly small amounts needed. Cobalt deficiencies in Australian sheep have been prevented with only 2 mg. of Co^{++} per year. Moreover, forage from pasture lands supporting healthy cattle and sheep can contain as little as 0.1 part of cobalt per million parts of dry matter. If the grasses contain less than 0.07 p.p.m., ruminants are likely to suffer. For some reason, pastures incapable of providing adequate Co^{++} for cattle or sheep maintain horses in perfect health. Either the ruminants themselves have higher requirements, or more probably the rumen bacteria have sizable needs of their own for proper growth. In marginal areas 10 g. of cobalt sulfate per 100 lb. of sodium chloride fed free choice is probably adequate. Since meat is an excellent source of B_{12}, man and the carnivores never show deficiencies except in human pernicious anemia, discussed on page 346.

Cobalt deficiencies in cattle and sheep have been encountered in Australia, New Zealand, Africa, Canada, parts of Latin America, Britain, Central Europe, Michigan, New England, North Carolina, and

Florida. As time goes on, diseases cured by cobalt will probably be studied elsewhere. Although the deficient pasture, hay, and fodder lands are scattered, the total acreage is large, and the stock losses are serious.

Symptoms of cobalt deficiency include gradual loss of appetite, emaciation, rough coat, scaly skin, reproductive failures, and especially anemia. Response to cobalt administration is usually rapid, and complete recoveries are common. Calves and lambs seem to be more susceptible than adult animals. In certain areas lack of cobalt is accompanied by a copper deficiency.

Cobalt is toxic when ingested to excess. Therapeutic doses of 30 to 40 g. per 100 lb. of stock salt are about the upper limit. Larger quantities are quite poisonous. Thus the quantity needed and tolerated for health lies in a poorly defined but fairly narrow range. Large doses of vitamin B_{12} do not seem to be harmful, and the excess is rather rapidly excreted in the urine. Some of the vitamin is eliminated in feces. There is no information on the excretion of other forms of cobalt.

Copper

This element is known to play several roles. It is a prosthetic group of the respiratory pigments called hemocyanins, serving as the principal oxygen-transporting proteins in the bloods of many marine forms, including crabs, lobsters, octopi, and snails. Copper is quite abundant in these and related species. In terrestrial animals copper is apparently distributed in smaller concentrations throughout all tissues. It functions in unknown ways to provide for absorption of iron from the diet and in the formation of erythrocytes. Furthermore, copper is a key component of several plant and animal enzymes and proteins, particularly the flavoproteins necessary to electron-transfer mechanisms. It circulates in the blood, mostly combined with protein, to the extent of about 0.0001 per cent.

Vegetables and meats contain sufficient copper for human requirements, and deficiencies are unknown. Milk is very low in copper, but fortunately the fetus accumulates this element during pregnancy. The newborn child has enough stored to last for a year or so and consequently does not suffer from the milk diet low in copper.

Plants grown on soils low in copper become deficient, and grazing animals may not receive enough available copper. Cattle and sheep seem to be the most susceptible domestic animals. The former are believed to need 50 mg. daily, and sheep and swine about 5 mg. From 5 to 8 p.p.m. of copper in feeds on the dry basis is enough to meet these requirements. In areas with deficient vegetation, salt licks containing 0.25 to 0.5 per cent copper sulfate prevent deficiency symptoms.

Since copper plays a diversity of roles in animals, lack of it leads to a variety of symptoms depending to some extent on the species and stage of maturity. Anemia is a general response, and depigmentation of the coat, retarded growth, bone disorders, diarrhea, abnormal hair growth and structure, and nerve malfunction are more or less common symptoms. Black sheep, for example, turn to gray, and all sheep regardless of color develop limp and shiny wool fibers. The animals find it difficult to walk, staggering and falling because muscle control is impaired by lesions of the nerves. Death can occur quite suddenly. When alternations of bones and nerves are not severe, administration of Cu^{++} is rapidly effective.

An excess of copper is dangerous, for Cu^{++} is fairly toxic. Hence the indiscriminate feeding of this element is risky. Sheep given 30 mg. of copper salts daily over an extended period develop hemolytic jaundice as a symptom of copper poisoning.

Excreted copper appears primarily in the feces, including the ingested but unabsorbed fraction. That eliminated from body tissues seems to be mainly lost to the intestinal tract by way of the bile. Urine is low in copper except in kidney disorders.

Fluoride

Although this anion has probably received more attention than any other trace element, its status is still much in doubt in most respects. In fact, there is still no conclusive demonstration that fluorine is essential to either animals or plants. One interesting recent experiment indicates that rats fed plants and yeast grown in "fluorine-free" systems grow more slowly than rats on the same diet supplemented with fluoride. Since the diets were not analyzed for fluorine to permit comparison with other studies, the experiment should be repeated with all analyses and controls. Other work using diets very low in fluoride gave erratic results or no signs at all of deficiency.

Whereas fluorine may or may not be a strictly essential element, there is no doubt about its occurrence in teeth, bones, and other body tissues. In the case of teeth, at least, F^- is beneficial when ingested at certain levels and markedly reduces the incidence of dental caries, especially in children. Apparently fluoride is most effective during the formative stages of teeth, but the protection conferred carries over to adulthood. In the laboratory, dental caries experimentally produced by feeding special diets are reduced or prevented when fluoride is included.

In certain areas the water supplies contain fluoride just sufficient for the protection of teeth. Many cities in the United States simulate the natural case by adding fluoride ion to the water to make the total concentration 1.0 to 1.5 p.p.m. The exact amount depends on the climatic conditions, which in turn control the quantity of water drunk. Topical application in the form of tooth paste containing sodium or stannous fluoride is recommended in areas without the anion in the water supply. Tea is a fairly rich source of fluoride, providing tea drinkers with an adequate supply. Unfortunately children do not profit from this source in most families. Sea foods are also relatively high in fluoride.

The mechanism of tooth protection by fluoride is unknown. Of two popular theories, one suggests that fluoride inhibits the acid-forming enzymes of oral bacteria; the other, that fluoride alters the structure of the enamel, making it more resistant to attack. Tooth decay is the only known deficiency symptom whatever the mechanism of action.

Insoluble fluorides are not absorbed and play no part in the diet. Soluble forms are readily absorbed, and the normal animal maintains a more or less constant body level, excreting the excess in the urine. Small amounts of fluoride occur in perspiration.

Water supplies containing more than 2 p.p.m. of fluoride produce mottled and stained teeth in human beings. At 5 p.p.m. the stains become prominent, and pitting and erosion are serious. Thus the optimum and toxic levels are quite close together, and the use of fluorides in foods and feeds requires careful control and supervision. Generalized poisoning is observed in adults in certain areas in Madras (an Indian province) where the water supplies may be very high in fluoride. The teeth are affected first, then the spine stiffens until the vertebrae fuse, and the rib connections become rigid. Ligaments and tendons calcify and emaciation proceeds until death results.

Farm animals are subject to the same problem of toxicity as are human beings. In both cases the natural foods contain fluoride, but the amounts present are invariably below dangerous levels. Fluorine in water is a serious factor for livestock as well as man. However, poisoning seldom becomes as drastic in animals, probably because their shorter life spans do not allow time for such prolonged ingestion and the consequent development of such severe symptoms.

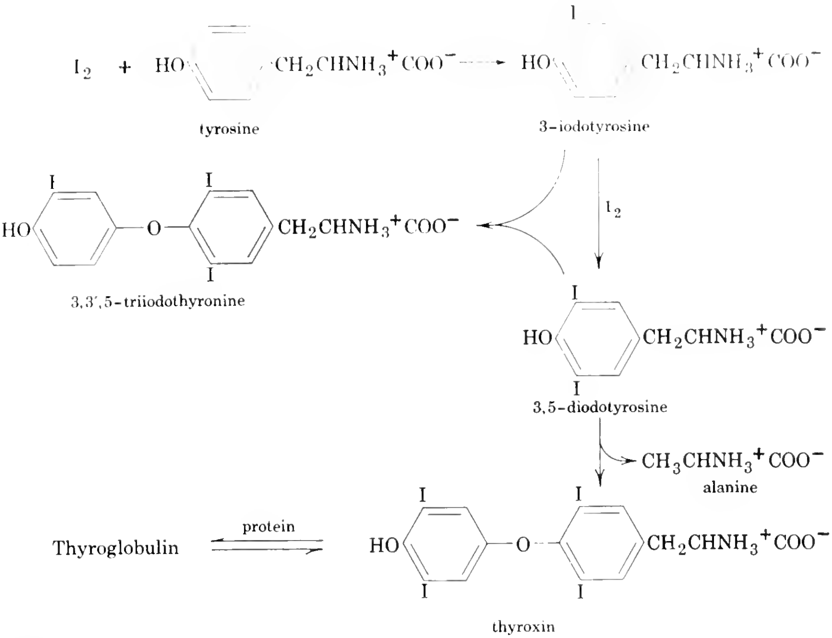
On the other hand, one special aspect of toxicity occurs only with animals. In order to provide diets with adequate calcium and phosphate, feeds are supplemented with bone meal or rock phosphate. The moderate concentrations of fluoride in the former are quite safe. However, the rock phosphates of North Africa and North America contain 3 to 4 per cent F^-, enough to provide 10 times the safe intake when included in feeds. The phosphate rocks of the Pacific and Indian oceans contain less fluoride but still enough to be potentially hazardous. The Association of American Feed Control Officials recommended in 1955 that the fluorine content of any minerals used in feeds be below 0.30 per cent for cattle, 0.35 per cent for sheep, 0.45 per cent for hogs, and 0.60 per cent for poultry. Furthermore, the amounts used should not raise the fluorine content of the total ration above 0.009 per cent for cattle, 0.010 per cent for sheep, 0.014 per cent for swine and 0.035 per cent for poultry.

It may require a year or more for symptoms of fluorine poisoning to develop in livestock. Then the teeth begin to deteriorate in young animals, bony projections appear on the skeleton, joints thicken, movement is slow and painful. Growth is poor and emaciation is common. Mortality of the young is high, not because they are poisoned but because the mother is physiologically impoverished. There is little transfer of fluoride from mother to offspring through the placenta or the milk. Hence the newborn and suckling animals suffer from starvation rather than toxicity. If fluoride poisoning does not begin until after the permanent teeth are fully developed, the teeth are not adversely affected and this group of symptoms is not observed.

Fluoride antagonizes certain enzyme activators and *in vitro* interferes with enzyme action. Enolase, for example, is a phosphoprotein and requires Mg^{++} for its activity. Fluoride inactivates the system by leading to magnesium fluorophosphate. Many of the enzymes depending upon calcium, magnesium, manganese, iron, copper, and zinc are potentially susceptible to the presence of F^-. The occurrence and extent of such inhibitions in the animal have been studied but little. According to one suggestion, fluoride interferes with the metabolism of ascorbic acid, and in another study the ion was found to affect the phosphatase of bone. A great deal more work is indicated.

Iodide

Although not known to be essential to plants, iodine occurs in them and is rather abundant in a variety of sea plants. Mammals all appear to need this element in small amounts and ingest it primarily as iodide ion, although organic forms will serve. Once absorbed, iodide is circulated to the thyroid gland in the throat, where much of it is concentrated and stored. Probably every body cell contains a little iodine, but 20 to 40 per cent of the total occurs in the thyroid. Once in this gland most of the iodide is oxidized to iodine, which initiates the following reactions:



Most of the iodine of the thyroid is stored as thyroglobulin, which acts as a reservoir for thyroxin and perhaps 3,3′,5-triiodothyronine. These last two compounds are hormones secreted by the thyroid gland and distributed by the blood. Both are active (with 3,3′,5-triiodothyronine the more powerful) in controlling the general level of metabolic activity by some unknown mechanism. A wide variety of enzymes has been proposed as sites of the action, but there is little agreement between investigators, and it is rather unlikely that all the diverse theories can be correct. Nevertheless, iodine deficiency, surgical removal of the thyroid, and goiter cause a marked reduction in the basal rate of metabolism. Iodine also occurs to a limited extent in a histidine derivative for which the function is not known.

Allowances are difficult to set and may vary with conditions. Estimates for man range from 60 to 300 μg. daily, and values calculated for chickens are 5 to 9, sheep 50 to 100, swine 80 to 160, and dairy cattle

400 to 800 μg. daily. Therapeutic doses are very much higher, but caution is indicated because an excess produces pathological changes in the thyroid gland.

In human beings a reduction in the rate of metabolism and enlargement of the thyroid gland are the most common symptoms of iodine deficiency. A serious human disorder called cretinism is attributed also to iodine deficiency, since it occurs with goiter and is much less common with iodide supplements in diets. General retardation of growth and mental development are typical, leading to dwarfed individuals with sharply limited mental capacity. Some response to administration of iodine and dried thyroid tissue is often observed, but recovery is never complete. Development of goiters is readily observed and has been correlated all over the world with low iodine contents of plants, soils, and water supplies. However, a few workers are coming to believe that something more is involved than a simple lack of dietary iodine.

The problem of endemic goiters is complex because they occur also in certain regions with iodine available in presumably adequate quantity. Furthermore, the incidence of goiter rises and falls rapidly without any known change in dietary iodine. For example, goiter was unknown in the early days of the frontier town of Edmonton, Alberta. Within one or two decades goiter became very prevalent and attracted much attention. Near-by settlements were without goiter until sometime later when it appeared in them and increased rapidly. The water supply was probably not a factor, since the towns compared all obtained their water from the Saskatchewan River. By about 1900, goiter had almost completely disappeared from Edmonton and its environs, but between 1910 and 1920 the disease again became widespread in the city without known changes in the food and water supplies.

During this last period goiter was very common in the Middle West and Northwest, and large-scale studies showed that the disease could be prevented in most individuals by supplements of iodide. Established cases could be controlled and sometimes cured by iodine therapy. As a result it is customary to add sodium iodide to salt for human and animal consumption. Sodium chloride thus supplemented is described as iodized. Since iodide ion is slowly oxidized and the iodine lost by evaporation, there is interest in using the more stable sodium iodate.

Although iodine deficiency can be experimentally produced, the absolute requirement now appears to be much lower than was once thought. Then how can goiters be explained? Some believe that natural thyroid antagonists occur in various plants like cabbage and turnips. Others suspect that dietary factors impede the absorption of iodide by the intestine. Still other workers suspect an epidemic or endemic infectious disease which impairs the normal function of the thyroid. Finally another opinion suggests that the intestinal flora may come to contain organisms trapping the dietary iodine or otherwise making it unavailable. All four schools of thought concur in the value of iodine supplementation as a means of overcoming the iodine deficiency developed in the presence of adequate iodine. Obviously the problem is a complex one.

Animals, like man, show diminution of metabolism when deprived of iodine, after removal of the thyroid gland, or by feeding of antithyroid (goitrogenic) compounds. Any of these treatments impedes the growth of young animals. Suspected retardation of mental development in human beings has not been universally found by different investigators but is probable in severe cases. In young animals and birds the reproductive systems develop slowly and perhaps incompletely.

Severe cases in animals are characterized by impairment of the central nervous system, for example, by reduced sensitivity to carbon dioxide and decreased rates of respiration. Apathy and decreased responses to stimuli are characteristic. Lack of sufficient thyroid hormone affects the endocrine system, leading to adrenals of small size. Skin becomes leathery and pale and loses flexibility. Hair is sparse and grows slowly. Pigs are born without hair. Feathers grow poorly, molting is inhibited, and species plumage patterns do not develop.

These symptoms develop in animals only on diets relatively much lower in iodine than the levels leading to human goiters, another puzzling factor. Feeds and forages from areas with little iodine in the soil are very effectively supplemented with iodized salt. This procedure is not invariably successful with human beings.

Iron

About 0.005 per cent of the animal body is iron, and perhaps two-thirds occurs in hemoglobin. Although present in small quantity compared to calcium, sodium, chloride, and others, iron is essential to the cells of animals. In addition to this role in hemoglobin, iron is a component of myoglobin, a protein of muscle that functions as an oxygen carrier. Myoglobin has the same prosthetic group as hemoglobin, but the protein moiety is different. Myoglobin has a greater affinity for oxygen than does hemoglobin and is believed to form a complex with oxygen that serves as a reservoir to meet oxygen demands for bursts of activity.

Iron is also a component of a number of enzymes, mostly of the group participating in the transfer of hydrogen and/or electrons. Thus the cytochromes, cytochrome oxidase, certain flavoproteins, xanthine oxidase, and others are iron proteins. Most if not all of these are essential.

Since most of the iron in animals occurs in the hemoglobin packed into the red cells, the state of this system tends to determine the over-all requirements. Studies with radioactive iron indicate that red cells normally have life spans averaging 127 days. When these cells disintegrate, the hemoglobin is also broken down and the iron released. However, though much of the organic material is excreted, nearly all the iron is retained and used again and again.

When iron is absorbed by the intestine it combines with a protein to form ferritin, a complex of ferric ion and phosphoprotein reaching 22 per cent iron. Ferritin accumulates in the spleen, kidneys, and especially the liver. Reduction of the iron to the ferrous stage releases it to the blood, which transports it to the bone marrow where new erythrocytes form. This storage and utilization system is efficient and little iron is excreted. Severe acute or chronic bleeding may reduce the iron reserves seriously, but losses are otherwise quite small.

Absorption of ingested iron is dependent to some extent upon the diet. Foods high in phosphate or phytic acid convert the iron to insoluble salts. Aside from this effect, iron allowances depend upon the physiological status. Infants have relatively high requirements of 200 mg./year, normally met in part by iron stored during the gestation period. At 2 years of age the need drops to half this value to rise during adolescence to a high of 350 mg. annually for boys and 500 mg. for girls. In males the requirement drops sharply to 80 mg. yearly but holds at about 300 mg./year for women with increased demands during pregnancy when the fetus begins storing iron. Liver, heart, eggs, yeast, sea foods, seeds, leafy vegetables, meat, poultry, and nuts are good to fair sources of iron. Milk, fats, white flour, polished rice, potatoes, and most fruits are poor sources. Ferrous sulfate is commonly used in supplementing deficient diets and during later stages of gestation. The efficient storage of iron in the body makes it unnecessary to consume iron regularly. A feast and famine sequence seems to work as well as any other regimen. Average American diets supply 15 to 20 mg. daily, an adequate amount even for child bearing.

Allowances for farm animals are difficult to set, but deficiencies are not common except perhaps in swine and laying poultry. Suckling pigs tend to be susceptible to iron deficiency, and 15 mg. daily has been recommended. The tentative requirement for chicks is 9 mg./lb. of feed with about 5 times that amount actually present in standard feeds.

Since an egg contains approximately 1.1 mg. of iron, laying hens sometimes do not receive enough of the element.

Milk contains little iron and the level is not raised by feeding iron to the mother. Therefore, most deficiencies in otherwise normal individuals occur in nursing young. The fetus accumulates iron, but prolonged nursing without other foods richer in iron than milk may allow exhaustion of the supply.

Physicians have claimed that lack of iron produces the commonest, clinically manifest deficiency disease of man. The dietary form is encountered in infants while that due to chronic blood loss occurs primarily in women. Anemia is invariably observed and is characterized by reduced numbers of red cells which are abnormally small and which are pale in color due to the reduction in iron. Simple iron-deficiency anemias respond quickly to the administration of ferrous sulfate. In more complex situations, copper or cobalt may also be necessary. Deficiencies due to lack of B_{12} or folic acid produce anemias but of the macrocytic or giant-cell type. In addition to this symptom of iron deficiency, others that may appear include any combination of listlessness, chronic fatigue, palpitation of the heart, lesions of the mouth and tongue, abnormalities of the finger and toe nails, and changes in the composition and appearance of the bone marrow.

The growth of pigs immediately after birth is unusually rapid, and when kept in concrete pens out of contact with the soil they cannot forage for the iron they need. Consequently piglets develop deficiencies rapidly when reared in modern quarters unless they are given iron. Feeding iron to the sow does not help since the element does not appear in the milk. The simplest effective treatment is provision of a trough of soil or sod for the pigs to eat. Anemia, lack of appetite, poor growth, and labored breathing are characteristic and often accompanied by a flabby appearance and edema of the head and forebody.

Iron anemia is not observed in poultry except perhaps during heavy egg laying and then of only mild type and probably not detrimental to the birds. Grazing animals develop anemias in certain areas in Florida where both soil and herbage are rather low in iron. However, it is now suspected that the trouble may actually be a deficiency of either copper or cobalt. Other than this possible case, herbivores seem not to develop iron deficiencies under field conditions.

Magnesium

This element occurs in animals in moderate amounts with about 75 per cent of the total in the teeth and skeleton. In these tissues magnesium occurs in complex salts with calcium and phosphorus with about a third of it available for other purposes when the amount ingested is insufficient. In addition to its structural role, Mg^{++} serves as an activator for a variety of animal enzymes. Well-known examples include all the kinases, transketolase, enolase, gluconolactonase, 6-phosphogluconic dehydrogenase, and at least some of the phosphatases. Although the details are unknown, Mg^{++} probably participates in the enzyme-substrate complex, perhaps as a linking agent.

Calves and chicks require enough magnesium to make approximately 0.05 per cent (dry basis) of the ration. Since feeds normally contain at least twice and usually three to four times this much, there is not likely to be a deficiency problem. The abundance of magnesium in both plants and animals almost automatically insures that herbivores as well as carnivores will be adequately supplied. Allowances have not been set except in the two cases mentioned. Green plants and bone meal are rich sources.

Naturally occurring deficiencies are unknown. The grass tetany (page 372) once thought to be a result of magnesium deficiency is now felt to arise from other causes. Experimental cases have been produced in rats, dogs, chicks, and calves. The characteristic symptoms are reduced levels of blood Mg^{++}, loss of the element from the skeleton, dilation of the blood vessels giving a flushed appearance, great excitability, convulsions, and death.

As in the case of calcium, the efficiency of absorption of magnesium depends upon the quantity of precipitating anion in the diet. Thus ingested magnesium is excreted to a variable extent. Otherwise Mg^{++} is lost through the kidneys.

Magnesium requirements, utilization, and excretion depend upon the levels of both calcium and phosphate. An excess of any one of the three can be antagonistic toward the others. A large dose of Mg^{++} tends to precipitate phosphate and lower its level below the normal physiological range. This effect thereupon interferes with the calcium balance and with calcification. Thus magnesium ion can be administered in toxic amounts, and the use of dolomitic limestone high in $MgCO_3$ as a Ca^{++} supplement is potentially hazardous unless adequate supplies of available phosphate are assured. Fortunately there is no problem of Mg^{++} toxicity with ordinary feeds and foods.

Manganese

This element is required for proper bone formation and for the development and function of the reproductive system. As is so generally true of the essential elements, manganese is necessary for the growth of the animal as a whole. Yet, although the needs are well established for these general roles, the mechanisms involved are quite unknown.

Perhaps the missing link lies in the activation of certain enzymes by Mn^{++}. Bone phosphatase, for example, needs this ion as a cofactor. Some decarboxylases, like those for oxalosuccinate and oxaloacetate, require manganous ion. Furthermore, many investigators believe that the manganese of liver is a component of arginase. Muscle adenosine triphosphatase, certain peptidases, and choline esterase may depend upon Mn^{++}. The possibility of participation in such diverse enzyme systems as these and others still unknown can account for the dependence of animals on this element. But we are still in the dark about the causes of deficiency systems. One known role of obvious importance played by manganese is that of the metal in the respiratory blood pigment of the shellfish, *Pinna squamosa.*

As is so often the case, the allowances needed by normal animals are difficult to set. Once again, ingested material is not all absorbed, and absorption varies with intakes of other ions, especially calcium, iron, and phosphate. Since most practical problems have been encountered with poultry, much work has been based on birds. As a result, the only firm estimates are those for birds and depend upon the breed and on intake of calcium, iron, and phosphate.

Both immature and adult chickens need feeds containing a minimum of 30 to 40 p.p.m. of manganese with the higher figure for heavier breeds, New Hampshire, Barred Rocks, etc. This level is just sufficient for normal growth of chicks, heavy egg production, hatchability, and the prevention of perosis or slipped tendon. Rations below this level should be supplemented with bran, middlings, or manganese compounds. The form in which manganese is added seems to be immaterial except that perhaps the phosphate is not absorbed. If the diet is particularly high in Ca^{++} and $PO_4^{\equiv}$, as with large supplements of bone meal, the above level of manganese is not sufficient and deficiencies occur unless the supply is increased. Fowl seem to have distinctly higher requirements than do mammals. Corn is a very poor source of Mn^{++} and is inadequate for poultry from this point of view.

Manganese deficiency is unknown in man although the element is presumably required. Tea, legumes, leafy vegetables, fruits, nuts, meats, and sea foods are good sources.

Deficiencies have been produced in rats, mice, rabbits, chickens, and pigs. The bones are poorly mineralized and those of the legs are deformed. Observed changes are microscopically different from those occurring in rickets. Reproduction is reduced or blocked with the effects ranging from production of poorly coordinated and partially paralyzed young to complete sterility of both males and females. Enzyme activity is reduced at least for liver arginase. Low manganese contents of certain soils in Holland are now held responsible for a disease characterized in dairy cattle by these same symptoms. Except in poultry other field deficiencies are unknown.

Poultry show the same experimental abnormalities and are much more prone to the deficiency disease. The condition is likely to become widespread when closely enclosed birds are fed rations mainly comprised of corn. In addition to the other symptoms, egg shells are thin and friable, eggs hatch poorly and embryos develop abnormally, and the Achilles tendon of the leg slips from its groove with crippling effect (perosis).

Manganese is not certainly known to be toxic since very large quantities have been fed. However, there is some indication of interference with the metabolism of iron. It is unlikely that manganese toxicity is a practical problem. Elimination of both excessive and normal amounts of manganese occurs by way of the feces, the manganese reaching the intestinal tract in the bile with which it is secreted.

Molybdenum

Although neither field nor experimental deficiencies in animals are known, molybdenum is believed to be essential to animals because this element is a required component of certain enzymes. Therefore, diets sufficiently low in molybdenum should give disease symptoms even though not yet observed. A deficiency is well recognized in plants, and molybdenum is known to participate in the fixation of atmospheric nitrogen. Perhaps the feeding of deficient plants might cause symptoms in animals. Apparently the latter need only minute amounts for xanthine oxidase and related iron flavoproteins.

Obviously neither allowances nor deficiency states can be described. Nevertheless, the effect of this element on animals has been much studied because of its toxicity. Unlike several of the other elements discussed, molybdenum is rapidly and effectively absorbed by the intestinal tract. Excretion is primarily urinary and occurs rather rapidly, so that poisoning is likely to occur only with regular ingestion of excessive amounts.

Toxicity of certain English pastures for cattle has been known for a century, and milder cases have been reported from California, Florida, Manitoba, and New Zealand. Less severe symptoms have been found in sheep, while pigs and horses show no ill effects in the areas with amounts disastrous for cattle. Areas toxic for cattle contain 0.002 per cent molybdenum or more in the surface soils, providing like concentrations (on a dry basis) in the forage from these fields. Plants with 0.0005 per cent (5 p.p.m.) molybdenum are harmless, but when the element is deliberately increased to the toxic range, the usual symptoms are observed. In cattle the symptoms include severe diarrhea, marked weight loss, disturbance of phosphate metabolism, reproductive impairment and failure, and bone and joint abnormalities.

Several aspects of molybdenum poisoning resemble the symptoms of copper deficiency. When this similarity was realized, it was discovered that feeding extra Cu^{++} prevents the symptoms of molybdenum toxicity. Thus an excess of molybdenum is antagonistic for copper and raises the requirement for the latter. In addition, experiments have shown that feeding sodium sulfate accelerates the excretion of molybdenum and raises the level which may be ingested safely. Similar results have been obtained in rats, where both copper and methionine are beneficial in alleviating the toxicity. Clearly, then, molybdenum poisoning depends upon the dietary levels of three elements, molybdenum, copper, and sulfur.

Phosphate

Phosphorus as organic and inorganic phosphates is, next to calcium, the most abundant "mineral" element in the animal. Of that present, 70 to 80 per cent is associated with Ca^{++} in bones and teeth. The remainder is dispersed throughout the soft tissues as organic esters like ATP, DPN^{+}, glucose-1-phosphate, phosphoproteins, phospholipides, nucleic acids, and inorganic phosphate. The roles of the phosphorylated metabolic intermediates are discussed in Chapter 9 on metabolism and Chapter 7 on energy. Some phosphoproteins are enzymes like pepsin, while others have different roles like casein, which in part supplies needed phosphorus to young animals. Phosphorus is structurally essential in nucleic acids and phospholipides. The roles of these compounds are discussed in other chapters. Inorganic phosphate is one of the reactants in many metabolic reactions and is formed in still others. None of the other elements discussed in this chapter plays such numerous and diverse roles as phosphate.

Human beings should receive approximately as much phosphorus as calcium with about 1 g. daily for adults and growing children. Slight increases are desirable during adolescence, the period of maximum growth. During the latter part of pregnancy 1.5 g. daily is recommended to meet the demands of the fetus, and 2.0 g./day is needed in lactation to provide replacement for that in milk. Seeds, legumes, meat, milk, and eggs are rich in phosphate. Hence almost any human diet contains enough and there is little concern over minimum daily allowances.

On the other hand, farm animals are not always so fortunate and tentative daily allowances have been set for them. Values for the different species and age groups are listed in the tables of the Appendix. Cattle should get from 3 to 20 g./day, depending on size, with special increments during lactation. Rations for sheep should contain 2.5 to 3.5 g. daily, increased to 5.0 g. for 150-lb. ewes in lactation. Swine requirements range from 5 to 10 g. daily with double the intake during breeding and lactation. Recommended allowances for chickens are from 0.60 to 0.75 per cent of the feed with about one-third of this as inorganic phosphate. Turkeys need somewhat more inorganic phosphate, 0.4 per cent of the ration, and a total of 0.75 to 1.0 per cent.

As suggested earlier in connection with both calcium and vitamin D, the minimum requirement for phosphate depends on other factors in the diet. The vitamin aids in the utilization of both elements and helps prevent deficiencies with borderline intakes of phosphate. However, an excess of Ca^{++} renders part of the ingested phosphate unavailable as the insoluble $Ca_3(PO_4)_2$. This condition is aggravated when the diet tends to raise the pH of the intestine. Carnivores normally have more acidic digestive tracts than herbivores and are less susceptible to phosphate deficiencies. The presence of sizable quantities of other cations like Mg^{++} may also reduce the availability of phosphate and raise the amount needed in the diet to compensate.

Phosphate deficiency is the most widespread of any in livestock. It occurs all over the world and has been reported in at least twenty states of the United States. Common in cattle, it occurs to a lesser extent in sheep when foraging on plants that do not supply enough phosphate. Soils may contain sufficient of the mineral for normal plant growth but not enough to raise the concentration in certain plants to the levels needed by animals. Minimal demands by animals probably exceed those of plants because of large amounts in the bones. Thus ruminants with their none too efficient absorption are prone to phosphate deficiency even in the feedlot unless supplementary phosphate is added. Note the caution necessary (page 378) when phosphate rock is used without removal of fluoride.

The symptoms of phosphate deficiency are loss of appetite and cravings for wood and bones, eaten instinctively in an attempt to overcome the shortage. Emaciation becomes severe and is accompanied by weakness, stiffness, and bone fragility with tendency toward numerous fractures of the pelvis and back. Less drastic deficiencies are hard to detect and may be common in highly producing dairy herds. Eventually the weight declines significantly, milk production falls, reproduction is impaired, and finally the more severe and obvious symptoms appear. Response to extra phosphate is usually quite good. Bran, cottonseed and linseed meals, milk, gluten, and soybeans are the best feeds from the point of phosphate content. Bone meal and phosphate rock (after reduction of fluoride to a safe level) are the most useful supplements in fortifying corn stover, hay, silage, and grains. Pastures can be made satisfactory by liberal application of phosphate fertilizers, which increase the content of this element in the plants and raise the bulk productivity of the pasture at the same time.

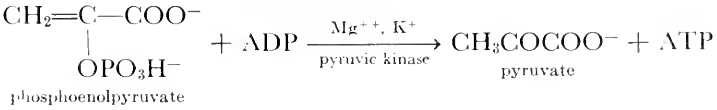
Large excesses of phosphate can be toxic in the sense that the absorption of most of the cations is impeded. This antagonism works both ways and has been mentioned in connection with calcium, iron, magnesium, and manganese. Herbivores excrete phosphate mainly in the feces, even the phosphate eliminated from the tissues. The carnivores excrete phosphate primarily in the urine, and man is intermediate. In addition, man and perhaps horses lose appreciable amounts in sweat.

Potassium

Some animals (rats, pigs, and chickens) have higher requirements for potassium than they do for sodium. In others the daily needs of K^+ are relatively lower. All animals need this element and use it in a variety of roles.

As an important ion in physiological fluids, K^+ enters into the electrolyte and water balances. Not so abundant as Na^+ in plasma, interstitial fluid, and urine, potassium ion is less of a factor than its close relative. However, K^+ is the more plentiful of the two inside cells and presumably is instrumental in the osmotic concentrations of most cells. For example, there is 6 times as much K^+ as Na^+ in muscle and more than 20 times as much in human erythrocytes. Clearly this concentration effect relative to sodium indicates that potassium performs somewhat different functions, although acting mostly in unknown ways.

Along with Mg^{++}, potassium is an activator for the metabolic enzyme pyruvic kinase in the glycolytic step. During muscular con-



$$\underset{\text{phosphoenolpyruvate}}{\begin{array}{l} \mathrm{CH_2{=}C{-}COO^-} \\ \quad\ | \\ \quad\ \mathrm{OPO_3H^-} \end{array}} + \mathrm{ADP} \xrightarrow[\text{pyruvic kinase}]{\mathrm{Mg^{++},\ K^+}} \underset{\text{pyruvate}}{\mathrm{CH_3COCOO^-}} + \mathrm{ATP}$$

traction, potassium is released extensively from the cells into the extracellular fluid. Apparently K^+ participates in changes in the actomyosin system. There is also displacement of potassium in the transmission of nerve impulses with rapid diffusion out of the cell during stimulation and return when the nerve is at rest. Some K^+ is needed with Mg^{++} to activate choline acetylase in the resynthesis of acetylcholine (page 308). Perhaps through its activity in the nervous system, potassium plays a part as a regulator in controlling the heartbeat, where K^+ increases and Ca^{++} decreases the rate. Both must be present and in the proper ratio for suitable performance of this organ. The individual effects are readily observed in tissue cultures of heart cells which show pulsations corresponding to beats.

Since plants contain far more K^+ than Na^+, daily allowances are of little practical concern. Rats, pigs, and chickens apparently need diets containing 0.2 to 0.3 per cent K^+ on a dry basis. Any ordinary diets adequate in other respects contain more than this proportion. Human beings ingest 2 to 5 g. of K^+ daily with perhaps half excreted as unnecessary.

Deficiencies are unknown in ordinary individuals on ordinary diets but can be produced experimentally. Poor growth and coat, kidney and heart damage, paralysis of the legs, and death have been observed in the laboratory. Potassium deficiency is observed clinically in connection with a variety of special pathological states and is automatically alleviated when the causative conditions are corrected.

Potassium toxicity occurs only in the terminal phases of uremia, Addison's disease, improper parenteral administration, and shock, or after severe burns. Otherwise, an excess of K^+ is quickly excreted, mainly in the urine. Lesser quantities are eliminated via the feces or perspiration.

The antagonism of K^+ for other ions involves Na^+, Ca^{++}, and Rb^+. Sodium and potassium levels are important and one ion exchanges for the other in nerve and muscle activity. During quiescent periods the reverse exchange occurs. An example of the antagonism between K^+ and Ca^{++} was mentioned above. Rubidium is rather toxic, but an increase in the K^+ counteracts the presence of the heavier ion.

Selenium

This element has been found essential for the formation of the enzyme formic dehydrogenase in *Escherichia coli.* Also, since it is accumulated in certain plants which grow only on soils of high selenium content, it may turn out to be essential for these plants.

Moreover, it was reported in 1957 that trace amounts of selenite ion can replace vitamin E and cystine in the prevention of degenerative liver disease in rats, degenerative diseases of liver, heart, kidney, and muscle in mice, and an exudative disease of chicks. Since selenite is more active on a weight basis than either of the organic compounds, the element may turn out to be essential in minute amounts. If, instead, tocopherol in physiological quantities completely prevents the development of symptoms, then selenium cannot be classed with the completely essential elements.

The physiologically active doses correspond to the levels of selenium in normal tissues and would be supplied by all ordinary diets. The actual requirements found experimentally are less than 1 per cent of the toxic levels and amount to about 4 and 10 μg. of selenium per 100 g. of ration for rats and chicks, respectively. Approximately 2 to 5 mg. of tocopherol per 100 g. of feed is needed for similar protection.

Though work on the utilization of selenium by animals is new and limited, there has been extensive and prolonged study of the toxicity of this element. Field cases have been numerous in the semiarid plains of the western United States with some reported in Alberta. The only other troublesome area known is a small region in Ireland. Symptoms of chronic poisoning appear when animals ingest plants containing 3 to 4 p.p.m. of selenium for prolonged periods. Consumption of plants with about 20 p.p.m. produces acute cases.

Plants accumulate the element from the soil, depending on the species and on the nature and pH of the soil. Large tracts of the plains and mountain states are high enough in selenium compounds to raise the vegetation to dangerous levels, including all the common grain and forage plants. Worse still, certain species, mostly belonging to a legume genus *Astragalus*, concentrate selenium up to several hundred to several thousand parts per million. These plants grow where the selenium level of the soil is high, where plant growth tends to be sparse because of limited rainfall, and where grazing is a logical industry from the viewpoints of terrain, soil, weather, and available moisture. At such levels acute poisoning is widespread, severe, and almost unavoidable.

Effective soil treatments have not been devised to remove the selenium or prevent its uptake by plants. Obviously use of hay or grain from such regions is quite dangerous, and extensive consumption of cereals high in selenium could be a serious hazard to human beings. However, vegetables take up but little of the element, milk contains only traces, white flour is rather less dangerous than the whole grain, and human diets as a whole seem by accident to contain less than toxic quantities. At any rate human cases of selenium poisoning have not been definitely identified.

Chronic poisoning in livestock is manifested by apathy and lack of vitality, emaciation, roughness of coat, anemia, lameness due to erosion of joints of the long bones, sore feet and partial loss of hooves, loss of long hair from cattle and horses and body hair from pigs, atrophy of the heart, and atrophy and cirrhosis of the liver. Acute selenium poisoning is characterized by blindness, partial paralysis, abdominal pain, excessive salivation, grinding the teeth, and death, usually by respiratory failure.

Adult fowl are not so drastically affected, but there is a reduction in food consumption, growth, and egg production. Hatchability is reduced, perhaps by levels too low to produce other symptoms in animals. The eggs are fertile but the embryos either fail to live to hatching or are grossly deformed monstrosities.

The mechanism of toxicity has not been identified. Rather obvious possibilities include interference with the metabolism of sulfur compounds and the replacement of the sulfur of enzymes. In the latter case the catalytic activity of those enzymes depending on —SH groups would be modified or destroyed.

Prevention and treatment are most unsatisfactory. Removal from or fixing of the selenium in the soil has not been accomplished. Feeding high-protein diets to some extent raises the level ingested without toxicity and ought to be of practical value where the diet contains borderline quantities of selenium. Administration of subtoxic levels of arsenic as arsenite or arsenate prevents selenium poisoning at the lower range of intake. In view of the potential hazard from this treatment, it is not very useful in the field. Thus no procedure can be recommended other than avoidance of the affected areas.

Sodium

This ion is closely associated with Cl^- in its ingestion, excretion, requirements, and most of its functions. Therefore much of the material discussed under chloride (page 373) is directly applicable here. In addition to its roles in salt and water balance, depending on osmotic concentration and control of protein solubility, sodium exchanges with potassium ions during nerve and muscle operation. Although the mechanism is in doubt, these functions are essential to animals. Furthermore, about a quarter of the Na^+ in the body occurs in the skeleton in an insoluble, rather inert form presumably necessary for the well-being of the animal.

Since plants contain much less Na^+ than K^+ and the requirements of animals are approximately the same for the two ions, vegetarians suffer sodium deficiencies unless provided with supplementary salt. Growth requirements for rats, chicks, pigs, and calves amount to about 0.1 to 0.2 per cent of the ration with the exact amount depending on the species and other factors in the diet. Recommended allowances and normal intakes both provide a safety factor with enough salt to make 0.5 per cent of dried ration. This amounts to about 0.2 per cent Na^+.

Excessive quantities are excreted by the kidneys as the chloride. Chicks seem to be rather sensitive, showing edema on diets containing 3 per cent salt. Other animals seem to tolerate much more. Na^+, Cl^-, and to some extent I^- have been added to human foods as condiments and as preservatives. Such general use makes deficiencies rare except in certain diseases and as a temporary result of prolonged and profuse sweating.

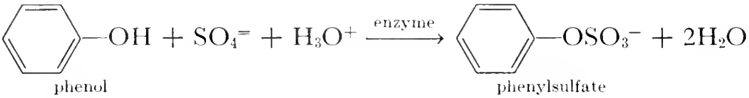
Sulfate

Although not normally discussed as an aspect of mineral metabolism, sulfur is required by all living cells. Much of this element occurs in the cellular amino acids, peptides, and proteins and is more properly covered under protein metabolism (Chapter 20). The sulfur in these compounds is of the organic sulfide or mercaptan type with a few derived compounds like taurine and a few heterocyclic substances like thiamine. Another group of biological materials not closely related to any of these is based on sulfate.

Many microorganisms assimilate sulfate ion and reduce it to intermediates converted into all the sulfur compounds needed by the organism. The higher plants operate the same way and are believed to take more sulfate from the soil than they do phosphate. Higher animals contain sulfate ion and excrete sulfur in this form, but early workers did not know that inorganic sulfate was important as such to mammals and birds.

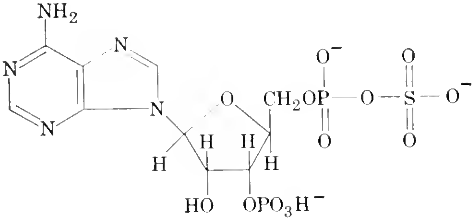
However, embryonic heart, bone, muscle, and artery tissues take up $SO_4^=$ and use it in tissue components. Of these components a major one is chondroitin sulfate (page 70), which serves with proteins as the cement substance of bone and cartilage. Since this material (also called chondroitin sulfuric acid although in the animal it would be present in salt form) is abundant in animals, the sulfate requirement during growth must be rather large. Perhaps much of the need is met by sulfate formed metabolically by oxidation of sulfide or hydrosulfide. Another member of the group, mucoitin sulfate (page 70), is also complexed with proteins in the mucosa of the gastrointestinal tract.

Most tissues of normal adult animals do not take up sulfate readily. However, during healing of injuries the ion is incorporated in large amounts and must be important. Furthermore, normal diets always contain small amounts of many potentially poisonous compounds. Some of these are detoxified by esterification with sulfate and excreted as the sulfate esters. Animals possess enzymes catalyzing this reaction, illustrated thus:



where the phenylsulfate appears in the urine with Na^+.

The sulfate reacting as shown above is first "activated" in that one or more intermediate compounds is formed with sufficient available free energy to drive the reaction forward. Active sulfate is believed to be analogous at least in part to ATP and to be a derivative of adenylic acid.



active sulfate

The need for sulfate in animal diets is uncertain, with reports both ways. Perhaps any requirement depends upon the developmental stage of the animal, the presence of wounds, the quantity of cations like Ca^{++} present that might precipitate sulfur compounds and $SO_4^{=}$, and the total sulfur intake. Nevertheless, when present, dietary sulfate is utilized. For example, hens transfer radioactive sulfur from sulfate ion to the albumen of their eggs. Hence, $SO_4^{=}$ may or may not be essential in the diets of animals, but it is important in animal metabolism and certain organic sulfates are essential animal components.

Zinc

Microorganisms and plants require zinc. In fact certain soils are so low in available Zn^{++} that the growth of plants is severely reduced.

However, animals consuming these plants are not affected, apparently because the actual zinc contents of the plants are not much below normal even though the plant cover is markedly reduced in quantity. Thus a requirement has been rather difficult to prove in animals.

First, it was demonstrated that zinc was an essential component of carbonic anhydrase. Since this enzyme catalyzes the exchange of carbon dioxide in the red cell and occurs in all higher animals, zinc must be essential. Other zinc proteins have been isolated, but proof that the zinc is not accidental has yet to be produced. The hormone insulin seems to be associated with zinc in the body. Some workers feel that the metal is involved in the storage and utilization of the hormone, but the problem is unsolved. Zinc is especially plentiful in marine species where it may play special roles, as in the snail *Sycotypus*, where zinc is the metal reported for the blood pigment corresponding to hemoglobin and hemocyanin.

Experimental zinc deficiencies have been produced in rats, pigs, and chickens. In the first two, growth is retarded, diarrhea and vomiting occur, and lesions of the skin, esophagus, and cornea appear. Chicks require zinc for growth and for bone and feather development. Current studies indicate that the required levels may be higher than previously suspected, perhaps above 0.01 per cent of the ration. The known foods and feeds contain enough zinc for all domestic and laboratory species, and zinc deficiency is probably not a practical problem.

Although Zn^{++} is not very poisonous, toxic levels can be reached with massive residues from crop sprays or industrial dusts. Human cases of zinc poisoning have resulted from prolonged storage and treatment of highly acid foods in galvanized containers. In general the symptoms are those of copper deficiency and may be alleviated by administration of Cu^{++}. Apparently a large excess of Zn^{++} impedes the utilization of copper. On the other hand, an experimental zinc deficiency in pigs was produced on diets high in Ca^{++}, so there must also be an antagonism between these two elements.

Incidental Elements

A number of elements are widely distributed in animals but are not known to play essential roles. As our knowledge increases, we may find that some of these actually are essential. However, an almost universal distribution in soils and plants may assure the presence of such materials in animals without any need for or utilization of them. Some elements play special roles in a few members of the animal kingdom without any known general function. Still others are included in this miscellaneous group because they are of practical concern as poisonous materials encountered in human affairs.

Aluminum seems to be universal in plants and animals without important roles in either and with no known function in the latter. Since diets free of aluminum have not been devised, one cannot say categorically that this element is not essential. Certainly there is no likelihood of a deficiency since all tissues contain it. Large quantities of aluminum interfere with phosphate utilization, but there seems to be no danger of this antagonism even when aluminum vessels are used in food processing.

Arsenic is also present in all soils, sometimes in large quantities. However, the levels taken up by plants are too small to harm animals. Soil concentrations may be high enough to poison plants, but the limited growth in such cases seems to be harmless to grazing animals. Residues from sprays and industrial dusts sometimes become toxic, and animal poisoning is occasionally reported. The introduction of other pesticides, in part replacing those based on arsenic, has reduced this problem.

Beryllium is a rare component of the earth's crust that finds industrial uses. Formerly, the element was employed in the phosphors lining fluorescent lamps. However, beryllium compounds from broken lamps prevented the healing of wounds. Moreover, inhalation of beryllium as dust leads to non-healing injuries of the lungs. Since the toxicity is persistent, use of the element in household lamps has been discontinued. However, metallurgical and other industrial applications still pose problems. Be^{++} is antagonistic for Mg^{++} and prevents activation of enzymes by the latter ion. Hence beryllium poisoning may be based on this sort of interference.

Barium is another rare element but, as distinguished from beryllium, is widespread and is normally found in bones. Barium also occurs in soft tissues, especially one of the layers of the eye of higher animals. The ion is quite toxic but except in traces is not encountered in soluble form outside the chemical laboratory or industry. One unconfirmed report states that Ba^{++} is essential for the proper distribution of calcium in the skeleton.

Boron is essential to a variety of plants and occurs in animals but without a known role. Although not particularly toxic because it is readily excreted via the kidneys, accidental ingestion of large quantities of boric acid or treatment of extensive burns with this compound will produce symptoms. Adult human beings normally ingest 10 to 20 mg. of boron daily without effect, so even accidental poisoning is very rare except in children who may mistake boron compounds for something like sugar and eat a large quantity.

Bromine is more abundant in living materials than iodine, the factor being 50 to 100 for animal tissues except the thyroid. Yet there is no indication that Br^- is essential, and extensive work has not produced a conclusive result. Dibromoindigo (royal purple) was a dye much sought by the ancients as a badge of prestige. This compound is found in small amounts in the snail *Murex brandaris.* Dibromotyrosine occurs in the skeletal proteins of certain other snails. Otherwise natural organic compounds of bromine are unknown.

Lead occurs in traces throughout nature without known function. Although the element is a cumulative poison, the quantities ingested by animals are normally safe. However, the prolonged use of lead sprays in orchards, for example, may increase the lead content of soils and hence of crop plants to levels that can cause slowly developing chronic toxicity. Lead poisoning has been diagnosed in small children from old dwellings painted repeatedly with lead paints. Apparently the children chew painted surfaces, perhaps during teething. Recent therapeutic methods are described as rapid and effective.

Nickel is another widespread element in animals without known function, although in this case little serious effort has been expended with modern techniques. The element is not particularly toxic, and poisoning is known only among workers in ore concentrating plants, where it presents a serious health problem.

Nitrates and nitrites can be ingested in dangerously high quantities. As mentioned earlier (page 325), water supplies occasionally contain toxic amounts. In addition, certain soils in semiarid regions or areas after intensive application of nitrate fertilizers may lead to high NO_3^- levels in particular plants. Oat straw, for example, may contain 5 to 10 per cent of potassium nitrate and produce trembling, staggering, rapid respiration, prostration, and death in cattle. Other plants are sometimes involved, and horses and sheep can also be affected. It is believed that poisoning is caused by the nitrite ion formed intestinally by reduction of the nitrate. Cases have been reported primarily from Wyoming, South Dakota, and western Canada.

Silicon as a universal component of soils is taken up by plants and is ingested by animals as an incidental element so far as is known. Silica is very abundant, amounting to 3 to 4 per cent of whole dried plants or 75 per cent of the ash of feathers. Toxicity is not observed except for the occasional formation of siliceous kidney stones in castrated male cattle or sheep.

Strontium is a widely dispersed but rare element associated with barium in bones. Some evidence suggests that Sr^{++} may be essential for the calcification of bones. Although soluble salts are quite toxic, natural levels present no problem. A special situation of potential concern is discussed below.

Vanadium occurs in minute amounts in plants, animals, and soils. It has been implicated in the development of bones and teeth, and if these findings are substantiated will be listed as an essential element in general animal nutrition. There is no problem of toxicity. Vanadium is the metal in the respiratory blood pigment of the group of marine animals called ascidians or *Tunicata*. Obviously it is an essential element for these species.

In addition to the normal elements, radioisotopes are important in nutrition and metabolism. They are widely employed in studying problems in these areas and are helping to add to our increasing knowledge. More directly, the contamination that follows the use of isotopes raises the levels ingested by animals and taken up by plants. This increase may ultimately become serious for two reasons.

First, the radiation produced by the decay of these unstable isotopes alters near-by molecules absorbing this radiation. Obviously the drastic change of sufficient numbers of molecules of critical kinds can have far-reaching biological effects. Second, the incorporation of certain unstable isotopes into biological molecules leads to destruction of these molecules when the isotope decays. In this case the atom changes into a different element, giving rise again to abnormal molecules.

These general considerations lead to concern over the slowly rising level of radioactive elements. Although immediate and acute effects are not expected except in specialized accidents, the biological future is in doubt. A major problem in animals is expected to be the isotope strontium-90, which accumulates and remains in bones. The biological retention of this element and the particular radiation emitted when this isotope breaks down make it a long-time hazard. Unfortunately, large quantities are formed during the explosion of hydrogen bombs and dispersed over the entire earth by atmospheric movements. Since relatively unknown and unmeasurable biological hazards must be measured against political imponderables, testing of hydrogen bombs is under heated debate. Although of concern, the explosion of nuclear weapons of other types does not present quite such serious immediate and long-term problems. Yet even the use of radioisotopes for medical and industrial purposes is a matter of real concern. Eventually reliable safety precautions, exposure levels, and preventive and therapeutic treatments will probably be worked out.

OTHER ESSENTIAL INORGANIC MATERIALS

Besides the elements and ions discussed above and the various organic compounds essential to animals, certain other inorganic materials are required. Some of these are available from both the environment and the metabolic reactions. Either source can serve. In at least one other case, namely oxygen, the animal does not develop useful quantities of the substance and depends almost solely on its environment.

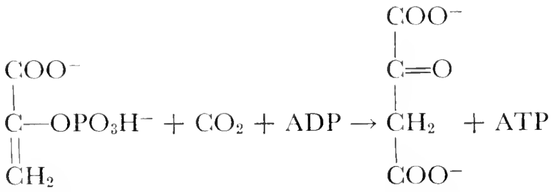
For the higher animals oxygen is the major terminal acceptor for electrons and protons and must be regularly available in quantity. Several tissues may temporarily function with a deficiency of oxygen. Muscle can accumulate rather large quantities of reduced compounds like lactate, but a limit is soon reached and these intermediates must be metabolized or tissue function ceases. Some tissues like brain are especially sensitive to oxygen lack, and investigators feel that each instance of oxygen deprivation causes permanent brain damage related in extent to the level of the deficiency. There is good evidence that an acute shortage of oxygen during certain critical stages of pregnancy can cause serious deformities of the offspring, human or animal.

Requirements for water are rather obvious but vary widely with species, activity, atmospheric temperature and humidity, and diet. Animals with extensive systems of sweat glands perspire even in cool weather. Evaporation of water is employed in hot weather to keep body temperatures near that normal for warm-blooded animals. Profuse sweating and panting lead to rapid loss of water and correspondingly raise the intake levels. Physical activity increases the rate of respiration and thus increases the total loss of water in the expired breath. On the other hand, loss of water is inversely related to the relative humidity, both lungs and skin surface being affected in animals with sweat glands.

In general, the larger the animal the more water required, with special adaptations in some species indigenous to arid regions. Many small desert animals are known to subsist for very long times without drinking. They have an efficient conservation system and depend on the moisture in their diets and the water formed by metabolic reactions. Individuals or species eating exceptionally moist foods should require correspondingly less water to drink. An even greater dietary effect occurs when the food is very salty, requiring a large volume of urine for excretion of the excess salt at a concentration compatible with normal function of the blood and kidneys. High-protein diets also tend to raise the water requirement because the excretion of urea is increased.

Since general daily requirements cannot be set, water is provided on a basis of free choice, and the animal allowed to suit himself when possible. In the higher animals there is a thirst-control center in the brain which produces the sensation of thirst or lack of it. The osmotic concentrations of body fluids are believed to activate the thirst response. Under rather average environmental conditions, a normal human adult has an obligatory loss of water amounting to about 600 ml. from the skin, 400 ml. in expired air, and 500 ml. in urine. If water intake, including drinking, the water of foods, and metabolic water, does not reach this total, the difference is supplied by dehydration of body tissues. The osmotic concentration of the body fluids rises and thirst begins.

The overall situation is less clear for carbon dioxide. Plants, of course, require this compound for photosynthesis and fix it in the organic materials of the plants. To a much smaller extent bacteria and animal tissues also fix carbon dioxide. It is now known that cultures of isolated animal tissues grow poorly or not at all in atmospheres free of carbon dioxide. Apparently this material is needed for important carboxylation reactions like the following:

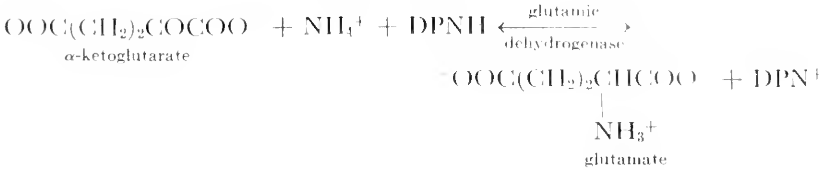


$$\begin{array}{lcl} & & COO^- \\ & & | \\ COO^- & & C{=}O \\ | & & | \\ C{-}OPO_3H^- + CO_2 + ADP & \rightarrow & CH_2 \quad + ATP \\ \| & & | \\ CH_2 & & COO^- \end{array}$$

phosphoenolpyruvate oxaloacetate

In normal intact animals the metabolic formation of carbon dioxide is a continuous process. A supply is always available in the tissues for reactions like the above. Hence, there is probably little need for carbon dioxide in the environments of most animals. However, since unicellular and other small forms would lose their metabolic carbon dioxide quickly by diffusion, they need an environmental supply.

In a somewhat similar way, ammonia as ammonium ion is required by animals. The synthesis of amino acids depends on the presence of this substance, which is utilized according to:



$$\underset{\alpha\text{-ketoglutarate}}{{}^-OOC(CH_2)_2COCOO^-} + NH_4^+ + DPNH \xrightleftharpoons[\text{dehydrogenase}]{\text{glutamic}} \underset{\text{glutamate}}{{}^-OOC(CH_2)_2\underset{\displaystyle NH_3^+}{\underset{|}{C}H}COO^-} + DPN^+$$

Again the green plants require NH_4^+ (or NO_3^-) from the soil, but the metabolism of animals provides enough in their tissues for such reactions. Undoubtedly complete removal of ammonium ion from the tissues would cause death of the animal.

ROLES OF ESSENTIAL ELEMENTS

Many of the special roles played by the elements in animals have been discussed above. Several of the general functions have also been mentioned without any real consideration of the roles themselves and the interplay of the factors involved. One of the physiological functions almost invariably depending on the presence of the essential elements is growth. It is clear that failure to grow is in reality due to a failure of some more specific process. Therefore, we are more concerned here with the specific problem than with growth, since satisfactory performance of the individual functions leads to the growth of young animals. Some of the biological processes depending primarily on one or a few elements have been summarized in connection with the elements concerned. Other processes or systems depending on more elements follow.

Skeletal Structure

Although there are several types of bone, most of the structure studies have involved the long bones. It is assumed that other bones are similar. Cells called osteoblasts lead to bone formation by complex processes yielding a combination of organic and inorganic compounds.

About 75 per cent of the volume of bone is organic material forming a matrix in which the inorganic fraction is embedded. This matrix is largely composed of collagen fibers distributed through the cement substance chondroitin sulfate. The collagen of bone does not seem to differ from that of other fibrous tissues. However, the cement substance of bone is somehow different from that elsewhere. The matrix is interspersed with osteoblasts and gives the bone its form and elasticity.

Microcrystals of inorganic material are distributed throughout the bone, contributing rigidity. The preponderant compound appears to be hydroxyapatite with the formula

$$[Ca(OH) \cdot Ca_4(PO_4)_3]_2$$

Since bone also contains magnesium, carbonate, citrate, and fluoride ions, substitution in this formula is presumed in order to account for the additional components. The sodium and potassium also present do not fit conveniently in place of calcium, and their locations are unknown. Since the chondroitin sulfate is a multivalent anion, all four major cations of bone may also be associated with it.

Practically every other cation of animals might be found in bone since most heavy metals form insoluble salts. Lead and radioisotopes have probably been studied the most in connection with their toxicities. In these and other abnormalities, the calcium is displaced. When the exchange is extensive enough, crystal structure changes, and the bone becomes hard and brittle or soft and flexible. Both conditions are potentially dangerous.

Many lower animals have exoskeletons of other types. A variety of these skeletons are predominantly organic matrices with calcium carbonate embedded (corals and shellfish). Crabs and the like possess skeletons composed primarily of chitin, a polymer of glucosamine (chitosamine). Most insects also have external skeletons containing 25 to 50 per cent chitin with other organic materials, mostly protein, making up the remainder. Obviously the chitinous skeletons are not inorganic and thus do not depend so directly on a supply of particular ions in the diet.

Electrolyte Balance

Cells cannot function properly in pure water or even in dilute aqueous solutions of essential substrates. Indeed, many cells are killed and even ruptured in such systems. If a suitable concentration of electrolytes is provided, the cell survives. This need is commonly expressed as a requirement for a particular osmotic concentration in the environment. An excess of electrolyte is equally unsatisfactory, so cells thrive only in characteristic ranges of osmotic concentration. In mammals and birds this corresponds to about $0.145M$ sodium chloride. Frogs and other cold-blooded animals operate at internal osmotic concentrations equivalent to about $0.111M$ sodium chloride.

Recall from Chapter 2 that osmotic pressure depends upon the total number of particles present. Since sodium chloride is dissociated into ions, each ion is effective and each contributes as much to the osmotic concentration as a molecule of glucose or any other undissociated molecule large or small. The intracellular fluid of a cell contains a wide variety of dissolved ionic and molecular materials. The osmotic concentration of the internal fluid is due to all of these solutes and corresponds to one of the sodium chloride concentrations listed above.

If a red cell, for example, from a sheep, is placed in distilled water, water passes rapidly by osmosis from the low osmotic concentration outside to the higher concentration inside the cell. This increase in liquid inside causes the cell to swell, disrupts its internal structure, and stretches the membrane to the breaking point.

On the other hand, a cell placed in a strong salt solution loses water by osmosis to the salt solution and shrinks. Serious shrinkage may permanently injure or kill the cell. Hence a rather close balance is required between the osmotic concentrations inside and outside cells. When the system is balanced, it is said to be isotonic, and any medium providing such balance is called an isotonic solution.

The nerve cells of animals are among the tissues that are especially susceptible to abnormal osmotic concentrations. As a result, appreciable changes or even prolonged slight deviations in the osmotic concentration of the interstitial fluid cannot be tolerated. This requirement means in turn that the osmotic concentration of the blood plasma must be very similar to that of the interstitial fluid and must hold constant. To achieve a degree of constancy in the blood, the effect of dietary fluctuations must be eliminated. The kidneys of higher animals can do this over a wide range by excreting practically salt-free urine when little salt is ingested and by excreting very salty urine when the diet is high in salts. Thus the proper functioning of the kidneys assures isotonic body fluids even though the diet varies widely in soluble salts.

Except for sodium and potassium salts, large quantities of soluble salts are seldom encountered. However, when they are ingested, such materials are rapidly excreted in soluble form in urine, as in the case of aluminum. Or the excess may be stored in the skeleton, as with calcium. Extra absorbed iron is stored in ferritin. Other elements may be excreted in the feces. Nickel, for example, to some extent passes through the intestinal tract without absorption. The remainder is absorbed but largely returns to the intestine for excretion. All these schemes play a part in preserving the balance of electrolytes throughout the body.

Gas Exchange

The soluble ions of the cells and fluids affect the utilization and elimination of gases. Aside from the elaborate system of gas exchange in the blood (see page 297) on which other tissues depend, several general influences are exerted by the ions present. First, electrolytes alter the solubilities of oxygen and carbon dioxide just by their presence. Since different salts have different effects on the solubilities, the composition of the solvent will make some difference. It will be recalled that blood and interstitial fluid contain different proportions of ions from those in intracellular fluid. Furthermore, physiological processes may change the composition of the liquid as in the diffusion of sodium and potassium ions in opposite directions through the cellular membrane during nerve function. Although these effects do occur, their importance to the organism is unknown.

Gas exchange is markedly affected by the pH, and pH is controlled by the dissolved ions. Primary and secondary phosphate ions ($H_2PO_4^-$ and $HPO_4^=$) are probably the most important for this purpose. Since chloride ion participates in the displacement of bicarbonate (a buffer ion) from cells, Cl^- is indirectly important also. Furthermore, formation of red cells depends upon the presence of several elements, iron, copper, cobalt, and zinc, which thus become indirectly necessary for gas transport since they are essential for the main gas-transferring mechanism.

Enzyme Action

Many of the essential elements are prosthetic groups of enzymes. Others function as less firmly bound cofactors but are still required for enzyme action. Although often unknown, the specific roles played are probably diverse. Some metals must actually participate in reactions as electron carriers and shuttle from one valence state to another as the electrons move through the substrate-enzyme-product system. In other cases, metals may serve to link enzyme and substrate, bringing the two together close enough and long enough for reaction. It is also suspected that the activating element may accelerate the reaction by helping to lower the energy of activation. Since a number of systems involve both cationic activators and organic coenzymes, the activator could be important in producing complexes that include the coenzyme and the enzyme. Thus the essential elements may be required by animals for one or more of the enzymatic functions as well as for skeletal, osmotic, or gas-transport roles. It is quite likely that most of the necessary elements have a multiplicity of functions.

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Feeds

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The animal must receive in its food all nutrients necessary for the construction and repair of metabolizing tissues and, in addition, sufficient oxidizable substances to furnish the energy required. Although the animal body is often compared with an internal-combustion engine or similar machine, there is at least one essential difference. When fuel is burned in an internal-combustion engine, heat and mechanical energy are produced from the chemical energy of the fuel. When food is eaten by the animal, the same phenomenon occurs but with the essential difference that the food also contributes to the manufacture of new tissues and to the repair of old ones, whereas fuel cannot contribute to new cylinders and pistons in the engine. Therefore the engine wears out and deteriorates, whereas the living machine is continuously being remade with new materials.

A complete food might be defined as one that contributes to every need of the body when this food is the sole article of diet for an appreciable period of time. It is impossible to find a single foodstuff that meets the exacting requirements of a healthy body. Perhaps milk most nearly approaches this ideal, but we shall find later that even milk may be lacking in a number of essential factors.

Most of the foodstuffs that we eat are deficient in many respects. It is for this reason that man has found by experience that dietary diversity encourages health and growth, whereas both suffer if one-sided diets are ingested for appreciable periods of time. Later chapters discuss the needs for the various food constituents and consider the fate of these substances during metabolism.

In this chapter we shall devote our attention primarily to the sources and chemical composition of some of our more important foods and feeds.

GENERAL COMPOSITION

When feeding stuffs are purchased on the market, pure food laws require that such feeds be sold on the basis of chemical composition. As a result it is customary to make the following chemical analyses on all feeding stuffs:

1. Moisture.
2. Crude protein.
3. Crude fat.
4. Crude fiber.
5. Ash.
6. Nitrogen-free extract.

Moisture

It is necessary to determine the moisture content of foods and feeding materials in order that we may compare the composition of one feed with that of another. As a rule feeds are usually fed and compared on what is known as a moisture-free basis in order that comparisons may be made on the basis of dry matter or total solids. Certain feeds deteriorate rapidly if the moisture content exceeds specific limits, which vary for different feeds. For these reasons knowledge of the water content is highly desirable. Furthermore a feed buyer cannot afford to pay feed prices for water. As a result the buyers of such commodities often prescribe the maximum water content.

Crude Protein

Since many proteins contain about the same amounts of nitrogen (16 per cent), it has become the practice of laboratories to determine the total nitrogen present in the feed and multiply this amount by the factor 6.25. This general factor is used for most proteins, although there are exceptions to this rule. For example, the factor 5.7 is used for the calculation of crude protein in wheat and its products, since the proportion of nitrogen in wheat proteins is greater (about 17.5 per cent) than in most other proteins. The nitrogen is determined by the Kjeldahl method, oxidizing the feed completely by boiling in sulfuric acid. This liberates the nitrogen in the form of ammonium sulfate. By the addition of a strong alkali (NaOH) the ammonia is liberated and is distilled into an acid of known strength. The amount of ammonia present is calculated after titration of the excess acid. This value is converted to nitrogen and thence to the amount of crude protein present. The term *crude* is used advisedly, since this method determines the total organic nitrogen, regardless of source. There are, of course, small amounts of nitrogenous compounds present in feeds which are not proteins. For this reason the term *crude protein* is applied.

Crude Fat or Ether Extract

When dry feeds are extracted with anhydrous ethyl ether, all ether-soluble materials dissolve. This extract, consisting mainly of glycerides of the fatty acids, is weighed after evaporating the ether and the percentage computed. Since the ether extract contains appreciable amounts of sterols, pigments, and other ether-soluble materials, the residue has been given the name *crude fat* or *ether extract.*

Crude Fiber

When a feed is boiled consecutively with sulfuric acid (1.25 per cent) and sodium hydroxide (1.25 per cent), the proteins, fats, and most carbohydrates are hydrolyzed, leaving, after filtration, the more resistant woody material (fiber). This is dried, weighed, and burned. The loss in weight on burning is assumed to be the indigestible organic portion of the feed and is known as "crude fiber." Although it is an index of the amount of indigestible matter in the feed, this designation is only approximately true for the reason that ruminating animals and horses can utilize appreciable amounts of crude fiber. Nevertheless it is the least valuable portion of the feed and for this reason should be kept at a minimum in feeds sold on the market as concentrates.

Ash

A weighed portion of a feed is ignited in a muffle furnace or over a gas flame until the carbonaceous matter is completely oxidized and the mineral salts remain in the crucible or dish as a white or grayish ash. This ash does not contain the mineral salts originally in the feed, since many of the salts are changed during the heating process. Organic substances containing such elements as sulfur and iodine are destroyed, and varying amounts of these elements may be lost by volatilization. If the temperature of heating is sufficiently high, it is possible that other elements such as sodium and potassium may be lost also. Usually, however, the temperature is controlled to prevent the volatilization of these ions. The ash of feeds contains the basic elements as oxides, carbonates, and phosphates, although these compounds may not have existed as such in the feed. Therefore, the ash determination is only a rough index of the true mineral content of the feed.

Carbohydrate (Nitrogen-free Extract)

The percentages of crude protein, crude fat, crude fiber, and ash on a moisture-free basis are totaled and subtracted from 100. This difference is the so-called "nitrogen-free extract" (N.F.E.), which is composed almost entirely of carbohydrates, although small amounts of certain organic acids and similar materials are included. For practical feeding purposes nitrogen-free extract may be considered synonymous with carbohydrates.

Regulation and Control of Composition

The Federal Food and Drug Act of 1906, revised in 1938, was enacted to protect the American public by regulating the manufacture and sale of foods, feeds, and drugs sold in interstate commerce. Standards for each type of commodity were established by law. Today most of the states in the United States also have enacted food laws controlling the purity and quality of foods and feeds manufactured or sold within their respective borders.

So far as laws relative to commercial feeds are concerned, all of them express feed composition in terms of moisture, crude protein, crude fat (ether extract), crude fibers, ash, and nitrogen-free extract, as described above. In order that the feed buyer may be protected, most feeding-stuff laws require that the manufacturer submit a guarantee regarding the chemical composition of the feed. If the feed-inspection laboratories find that a product does not conform to the guaranteed analysis, the manufacturer and the dealer are subject to legal action.

Many animal feeds differ in chemical composition from those foods commonly used for human consumption. This is due to the fact that domestic animals are capable of utilizing many products that the human digestive tract cannot.

FEEDS OF PLANT ORIGIN

Feeding stuffs may be classified from the standpoint of source of material, beginning with the feeds obtained from plant sources.

Grains or Cereals

The cereal grains and their by-products form the greater part of all the concentrated feeds used for livestock.

Corn is by far the most important crop in the United States. About one-fourth of our crop acreage is planted to corn and 85 per cent of it is harvested for grain. The remainder is used for silage. Wheat ranks second to corn in acreage, but most of it is converted to flour and other human foods. Only a small portion, mostly poorer grades, is used directly for livestock feed. Oats ranks third in acreage among the cereals but is second to corn in importance for stock feed. Barley is fourth in cereal production. It is an important feed crop in both dry areas and those with short growing seasons, conditions which are not conducive to good corn yields. Sorghum and millet are also grown in the plains states in regions of low rainfall, since they are more drought resistant than corn.

Cereals such as wheat, corn, barley, oats, sorghum, and millet are high in carbohydrates (N.F.E.) and low in crude fiber (Table 16–1) and, therefore, are valued as supplements to the hay and fodder components of the animal ration. These cereals are rich in total digestible nutrients and net energy and in addition are very palatable to livestock. Grains are relatively low in both protein content and protein quality, being generally deficient in lysine, tryptophan, and methionine (Chapter 5). In feeding poultry and swine, these deficiencies must be corrected by supplementing with high-quality proteins for efficient production.

TABLE 16–1. Per Cent Composition of Some Typical Cereal Grains

Grain	Dry Matter	Ash	Crude Protein	Crude Fiber	Crude Lipide	N-free Extract
Corn, dent No. 2	85.0	1.2	8.7	2.0	3.9	69.2
Wheat	89.5	1.9	13.2	2.6	1.9	69.9
Barley, feed	90.3	4.1	13.5	8.7	4.1	60.5
Oats	90.2	4.0	12.0	11.0	4.6	58.6
Sorghum	89.6	2.0	10.8	2.3	2.8	71.7
Millet (Proso)	90.4	3.3	11.9	8.1	3.4	63.7

Cereal grains are also low in minerals, particularly calcium. Animals receiving roughages get adequate amounts from such sources. The phosphorus content of grains is not high, but the levels are more nearly adequate than in the roughages.

Cereals are poor sources of most of the vitamins. Yellow corn does supply appreciable quantities of the pro-vitamin A. Cryptoxanthine and vitamin E are present in fair amounts. The remaining vitamins must be obtained from other sources.

In addition to the whole grains, factory by-products such as brans, middlings, gluten feeds, and distiller's grains are sold as ration ingredients. The compositions of some representative products are shown in Table 16–2. These by-products are better protein sources than the original grains but are lower in carbohydrates (N.F.E.). Because of the higher protein content, these grain by-products are used frequently as protein supplements. The protein quality does not equal that of the oil seeds or animal products but is satisfactory for the feeding of ruminants. The nitrogen-free extract contains greater quantities of pentosans and lower quantities of starch than do the cereals.

TABLE 16–2. Per Cent Composition of Some Cereal By-products

Feeding Stuff	Dry Matter	Ash	Crude Protein	Crude Fiber	Crude Lipide	N-free Extract
Brewer's grains	92.9	3.6	27.6	14.3	6.5	40.9
Corn gluten feed	90.9	6.3	25.5	7.6	2.7	48.8
Distiller's corn grains	92.9	2.5	28.3	11.4	8.8	41.9
Distiller's corn solubles	93.0	7.4	26.7	2.6	7.9	48.4
Winter wheat bran	89.9	6.2	15.5	8.9	4.2	55.1
Wheat middlings	89.7	4.5	18.0	7.4	4.7	55.1

Additional by-products not listed in Table 16–2 but sold as cattle feed include oat-mill and clipped oats by-products. Oat-mill feed includes the hull and fragments of the kernels. It has a feeding value of about 30 to 40 per cent that of corn. Oat clippings have a higher feed value. They are used chiefly as an ingredient in mixed feeds.

Meals from the Oil-seed Industry

Cottonseed, flaxseed, and soybeans are three important oil-containing seeds used in livestock feeding. Peanuts, coconuts, safflower, and sunflower seeds are available in lesser quantities in the United States, although in certain areas of the world each one functions as a major source of oil-seed meal. As a rule oil seeds are seldom fed before extraction because of their high content of oil and in some cases their high fiber content. The oils are removed by pressure or extraction, and the by-products are sold in the form of cake, meal, or pellets for

TABLE 16–3. **Per Cent Composition of the Common Oil Meals**

Feeding Stuff	Dry Matter	Ash	Crude Protein	Crude Fiber	Crude Lipide	N-free Extract
Soybean meal (solvent process)	90.4	6.1	45.7	5.9	1.3	31.4
Cottonseed meal (hydraulic) (Texas analyses)	92.6	5.6	42.1	10.5	6.1	28.3
Linseed meal (solvent process)	91.0	5.8	36.6	9.3	1.0	38.3
Peanut meal (hydraulic) (hulls)	92.3	4.8	41.1	15.0	6.6	24.8
Safflower (hydraulic) (hulled)	90.5	6.4	42.5	8.5	6.7	26.4
Sunflower (hydraulic) (hulled)	94.3	5.9	49.5	5.4	4.9	28.6

feeding purposes. These meals (Table 16–3) are characterized by their high protein contents and are important as concentrates for supplementing the normally lower protein levels of grain rations.

Soybean meal or cake has become our most important high protein supplement. Total production exceeds 5,000,000 tons annually, more than twice the production of cottonseed meal. Heated soybean meal is an excellent protein supplement for most forms of livestock, but the raw meal is not used very efficiently by non-ruminants because of the presence of a trypsin inhibitor (page 146). Soybean protein possesses superior biological properties, owing to a better distribution of essential amino acids than is found in most plant proteins. Soybean meal is low in calcium and lower in phosphorus than are cottonseed meal and the wheat by-products.

Cottonseed meal is a by-product of the cotton industry, which occupies an important economic place in the southern United States. Cotton is grown for the fiber, but the cottonseed meal and cake, secured in the production of cottonseed oil, furnish a protein supplement second in value only to soybean meal. The production of meal averages 2,500,000 tons annually. Cottonseed meal contains a toxic substance called gossypol, which varies in amount, depending upon climatic and soil conditions. Cattle are able to ingest large amounts of cottonseed cake without showing ill effects, but other types of livestock are more susceptible. Fortunately much of the gossypol present in cottonseed is inactivated by the heating processes employed during the oil extraction and solvent stripping of the meal. Gossypol can also be inactivated by treatment with chemicals such as ferrous sulfate. It is now thought that many previously reported cases of cottonseed meal poisoning in cattle were caused by dietary deficiencies, largely vitamin A deficiency, rather than gossypol toxicity. Cottonseed hulls are also fed to animals. They are high in fiber and therefore replace a portion of the roughage in the animal ration.

Linseed meal, a by-product of the flaxseed crushing industry, is a third important source of protein for livestock feeding. About 100,000 tons of linseed meal is used annually as a supplement for ruminants. This protein source is not an effective protein supplement for non-ruminants since it contains a toxic factor, but it is a good source of calcium and phosphorus. Linseed meal is highly regarded in fitting cattle for show or sale, for it aids in the production of a sleek hair covering. It also has a laxative effect because of the high mucilage content of the hull. This cathartic action is desirable when animals are fed non-leguminous roughage.

Peanuts are an important crop in the southeastern United States, but most of the nuts are sold for human consumption or foraged by pigs in the field. Peanut meal, when available, is a good protein supplement for most livestock. Safflower meal from carefully hulled seeds is a good supplement for ruminants. Because of methionine and lysine deficiencies, it is not satisfactory as the sole protein addition to poultry and swine rations. Sunflower seed meal is also deficient in lysine. These meals are used to only a limited extent because of the small supplies available.

Hay and Fodder Crops

In the fresh green state these feeds contain large amounts of water and small amounts of dry matter. After drying they are characterized by relatively high amounts of crude fiber and ash with small amounts of soluble carbohydrates. The protein content varies with the type of plant. High-grade alfalfa and other legume hays are quite rich in protein of good quality (Table 16–4). The stage of maturity of the crop and the curing conditions are important factors in determining the quality of legume hay. Most of the nutrients are concentrated in the leaves so any treatment which leads to loss of this portion of the plant lowers the feed value of the hay. In humid areas where field curing is frequently complicated by rains, dehydration either in the field or in stationary dehydrating plants is used extensively for the preparation of high-quality alfalfa meal for poultry and swine rations. This method is generally considered too expensive for average farm use for the preservation of roughage. In recent years considerable

TABLE 16–4. Per Cent Composition of Dried and Ensiled Fodder Crops

Feeding Stuff	Dry Matter	Ash	Crude Protein	Crude Fiber	Crude Lipide	N-free Extract
Hay						
Alfalfa hay	90.5	8.0	15.3	28.6	1.9	36.7
Alfalfa meal (dehydrated)	92.7	10.1	17.7	24.0	2.5	38.4
Clover hay (red)	88.3	6.4	12.0	27.1	2.5	40.3
Sweet clover hay	90.7	7.5	13.5	30.2	1.9	37.6
Corn stover	90.6	5.8	5.9	30.8	1.6	46.5
Timothy hay	89.0	5.0	6.6	30.3	2.3	44.8
Prairie hay (western, mature)	91.9	7.5	4.4	30.9	2.6	46.5
Oat straw	89.8	6.3	4.1	36.3	2.2	40.9
Silage						
Alfalfa-molasses (wilted)	35.8	3.1	6.0	10.9	1.1	14.7
Grass-legume-molasses (wilted)	33.8	2.2	5.1	8.6	1.3	16.6
Corn (well matured)	27.6	1.6	2.3	6.7	0.8	16.2
Sorghum	29.7	2.3	2.5	7.4	0.8	16.7

quantities of legumes and other forages have been preserved by ensiling, either in upright or in trench silos. The losses of nutrients are decreased and β-carotene is preserved to a greater extent than under the best haying conditions. It is necessary to add a source of carbohydrate such as ground barley or molasses to provide rapid fermentation or to add acid or bisulfite preservatives when a low-carbohydrate forage is ensiled. Although legume and grass silage have become popular in recent years, corn and sorghum silages continue to occupy an important position in the feeding program.

Grass

Pasture furnishes the foundation for efficient livestock production. In recent years a shift in emphasis towards "grassland farming" has taken place. This shift has occurred partially because of increased attention to water conservation and erosion prevention and partially because of the realization of the value of good pastures in livestock production. Young, actively growing grass contains more protein than good-quality alfalfa hay. A mixed legume-grass pasture furnishes even greater amounts of good protein. Animals feeding on adequately fertilized pastures require little or no protein supplements. Grain supplementation is required, however, for high milk production and a good finish on beef cattle.

Vegetables

In some parts of Europe, root crops are raised as feed for livestock. Crops such as mangels, sugar beets, rutabagas, and turnips are sometimes fed as sources of carbohydrates in the animal ration. Pumpkin, squash, cabbage, and kale are sometimes fed for their β-carotene contents. Such use is insignificant in comparison with that of the forage and seed crops discussed above. Vegetable tops, leaves, and other wastes are utilized for livestock feed in the immediate neighborhood of canneries. Beet pulp, the residue remaining after extraction of beet sugar, is dried and used directly or in combination with molasses as a carbohydrate supplement in dairy feeding. Beet tops are dried or ensiled and fed as roughage in animal production. Vegetable residues tend to be low in protein but furnish minerals, carbohydrates, and in many cases carotene.

Molasses

Molasses is a by-product of the manufacture of sugar from cane and beets. Molasses-type syrups are also recovered from cannery wastes and residues of citrus and other fruits. Such syrups supply some protein and minerals and considerable quantities of soluble carbohydrates but are valued chiefly because they increase the palatibility of other components of the animal ration. Molasses is also a good dust inhibitor in ground mixtures and is a good binder in making pelleted feeds. The use of molasses as a silage preservative was mentioned earlier.

FEEDS OF ANIMAL ORIGIN

Feeds of animal origin can be classified into two large groups: (1) those consisting of milk or milk by-products, and (2) those consisting of packing-house by-products.

Milk and Milk By-products

Before World War II milk products and packing-house by-products were available in quantities and at prices which permitted the inclusion of significant amounts in mixed feeds. With the development of better methods of dehydration much of the surplus skim milk is now dried and sold for human consumption. Condensed and dried buttermilk, whey, and lower-quality dried skim milk are still used extensively in poultry mashes. Whey, as a by-product of cheese making, is also a feeding material, but its consumption has been confined mainly to hogs and poultry.

Milk is the most nearly complete food known to science. It is the normal secretion of the mammary gland (page 324). Although there are many kinds of milk (Table 16–5), all references here, unless otherwise indicated, will be to cow's milk.

TABLE 16–5. Per Cent Composition of Milk from Different Species

	Water	Casein	Albumin	Fat	Lactose	Ash
Human	87.41	0.91	1.23	3.76	6.29	0.31
Cow	87.27	2.95	0.52	3.66	4.91	0.69
Goat	84.14	3.04	0.99	6.00	5.02	0.81
Sheep	81.90	4.57	1.26	6.52	4.82	0.93
Camel	87.04	3.49	0.40	2.76	5.57	0.74
Buffalo	82.14	4.29	0.49	7.44	4.81	0.83
Horse	90.68	1.27	0.75	1.17	5.77	0.36
Ass	89.88	0.73	1.31	1.50	6.09	0.49
Reindeer	68.20	8.40	2.00	17.10	2.08	1.50

Cow's milk varies in composition, particularly in fat, depending on the breed of cow from which the milk is obtained (Table 16–6). Other factors, such as inheritance, individuality, time of milking, and period of lactation, also influence the composition of milk.

TABLE 16–6. Effect of Breed on Milk Composition

(Expressed in Per Cent)

Breed	Water	Protein	Fat	Lactose	Ash	Total Solids
Jersey	85.27	3.80	5.14	5.04	0.75	14.75
Guernsey	85.45	3.84	4.98	4.98	0.75	14.55
Ayrshire	87.10	3.34	3.85	5.02	0.69	12.90
Shorthorn	87.43	3.32	3.63	4.89	0.73	12.57
Holstein	88.01	3.15	3.45	4.65	0.68	11.93

Although it is possible to affect the amount of milk produced by underfeeding and overfeeding, it is not possible to produce marked changes in the fat, protein, or sugar content of milk by changes in feeding practice. It is possible, however, within narrow limits, to change the nature of some of the ingredients. For example, certain feeds tend to yield butterfat which varies in its melting point, but efforts to increase the percentage of butterfat by feeding methods have not met with commercial success.

Examination of Table 16–6 on milk composition shows that the total solids of cow's milk consist largely of butterfat, proteins, lactose, and inorganic salts. Milk also contains carotene, vitamins A and D, riboflavin, thiamine, and ascorbic acid.

From a nutritional standpoint the proteins of milk are of great importance since they contribute essential amino acids necessary for normal health and growth. The proteins of milk are casein, lactalbumin, and lactoglobulin. Casein is present in cow's milk in the largest amount and represents that portion of the milk which precipitates when milk becomes sour. This precipitate is called the "curd" and is marketed as cottage cheese.

Lactalbumin is a water-soluble protein which is coagulable by heat; although it rarely occurs in excess of 0.5 per cent of the milk, it constitutes about one-sixth of the total milk protein. Lactoglobulin is a water-soluble protein which exists in normal milk in very small amounts.

Butterfat is a highly palatable mixture of glycerides of fatty acids. The unique flavor of butterfat is attributed in part to the presence of diacetyl (dimethylglyoxal) which is formed during the ripening of the cream. In addition, branched-chain aldehydes of 5 to 10 carbon atoms contribute to the flavor. The unique physical properties of butterfat result from the types of fatty acids combined as glycerides. Butterfat is characterized by the presence of a relatively large amount of short-chain fatty acids such as butyric, caproic, caprylic, and capric acids.

From the viewpoint of nutrition it is unfortunate that the market value of milk is based solely on its butterfat content. From an energy standpoint butterfat has no advantage over several other edible fats and oils. Butterfat does make important contributions to the vitamin A content of the diet, but the carotene and the vitamin A contents of butter are subject to wide seasonal variations. Vitamin A can be obtained more economically from other natural foods, particularly leafy vegetables, eggs, and whole milk.

The chemistry of lactose has been discussed in Chapter 3. This sugar is not hydrolyzed by adults as efficiently as other sugars, although infants and young animals hydrolyze lactose to glucose and galactose quite efficiently. Lactose forms lactic acid in the intestine, thereby favoring the development of desirable types of acid-forming organisms which discourage the growth of putrefactive organisms. Lactic acid also promotes the utilization of calcium and phosphorus by increasing the intestinal absorption of these important mineral elements.

Milk characteristically contains mineral salts in approximately the same proportions as in the body of a newborn animal. Milk ash contains potassium, calcium, sodium, magnesium, phosphorus, chlorine, and sulfur, as well as traces of copper, iron, zinc, aluminum, manganese, and iodine. Citric and carbonic acids are important, combining with bases to form salts. The other anions of milk are chloride and phosphate.

Lecithin and cholesterol are also normal milk constituents. The former ranges from 0.03 to 0.05 per cent whereas the latter fluctuates with the fat and may vary from 0.01 to 0.02 per cent.

TABLE 16–7. Approximate Composition of Some Important Milk Products

(Expressed in Per Cent)

Product	Water	Protein	Fat	Lactose	Ash	Sucrose	Salt	Lactic Acid	Curd
Powdered whole milk	2.00	26.91	28.65	36.50	5.94	...	...	...	...
Evaporated whole milk	73.63	6.71	8.22	10.13	1.55	...	...	...	...
Sweetened condensed milk	27.03	7.85	8.99	12.65	1.76	41.65	...	...	...
Whole milk	87.30	3.55	3.62	4.82	0.71	...	...	...	...
Light cream	72.46	2.95	20.00	4.00	0.59	...	...	...	...
Heavy cream	63.41	2.58	30.00	3.50	0.52	...	...	...	...
Whipping cream	54.35	2.21	40.00	3.00	0.44	...	...	...	...
Skim milk	90.36	3.72	0.15	4.98	0.80	...	...	...	...
Evaporated skim milk	71.05	11.16	0.45	14.94	2.40	...	...	...	...
Powdered skim milk	3.89	35.42	1.74	48.74	8.08	...	...	...	...
Butter	13.90	...	82.41	...	...	...	2.51	...	1.18
Buttermilk	91.61	3.30	0.50	3.40	0.65	...	...	0.50	...
Condensed buttermilk	60–64	12–15	1–2	16–20	2.5–3.5	...	...	2–3	...
Dried buttermilk	1.93	38.74	5.87	39.91	7.68	...	...	5.87	...
Cheddar cheese	37.33	23.39	33.41	...	7.02	...	...	...	...
Domestic Swiss cheese	30–34	26–30	30–34	...	3–5	...	1–1.4	...	...

Table 16–7 shows the approximate chemical composition of some of the more important products made from milk.

Packing-house By-products

By-products of the meat-packing industry have been used extensively as protein supplements in rations for domestic livestock, and, in spite of the present high cost of these materials, they are still considered essential for successful feeding of swine and poultry. Concentrates of this type (Table 16–8), which are most commonly used, include tankage, meat meal, meat scraps, blood meal, and pork cracklings. During the past few years the prices of tallows and greases have been depressed to the point where their inclusion in the animal ration has become economically feasible. Such fats reduce dustiness and in many cases improve the texture and palatability of the feed. In beef-feeding trials incorporation of stabilized fat up to 5 per cent produced efficient gains. Addition of 5 to 10 per cent fat to poultry rations produced good gains and superior finish in broilers. Such fats must be stabilized with antioxidants to prevent the development of rancidity.

TABLE 16–8. Per Cent Composition of Some Typical Packing-house By-products

Feeding Stuff	Dry Matter	Ash	Crude Protein	Crude Fiber	Crude Lipide	N-free Extract
Blood meal	92.2	4.7	84.7	1.1	1.0	0.7
Fish meal	92.9	17.6	63.9	0.6	6.8	4.0
Meat scraps	93.9	25.4	55.8	2.1	9.3	1.3
No. 1 tankage	93.1	20.2	60.6	2.0	8.5	1.8

Fish meal and ground dried fish are made from waste materials from fish-packing plants or from types of fish that are not acceptable as human food. Fish products are valuable as protein supplements in many types of livestock rations and as sources of easily available calcium and phosphorus because they contain appreciable amounts of ground fish bone. Marine fish are also rich in iodine.

Packing-house by-products and fish meals are valued primarily for the biological value of the animal protein they contain. Animal proteins are biologically superior to plant proteins because they contain better combinations of the essential amino acids required by animals for normal growth and reproduction.

Packing-house by-products are generally used only for poultry and swine. There is no advantage in using such protein supplements in bovine or sheep rations unless they can be purchased at a lower price than plant proteins. The superior biological value of animal proteins constitutes no advantage for ruminants since the microorganisms of the latter animals supply the essential amino acids.

ANTIBIOTICS AND VITAMINS

The discovery in 1949 that antibiotics added to the ration can increase the rate of growth of normal chicks and young pigs opened up new vistas in livestock feeding. Since that time numerous experiments have been conducted with a variety of antibiotics. The antibiotics most commonly used include aureomycin, terramycin, stabilized penicillin, and bacitracin. Some of the other antibiotics have little or no effect. The cause of the growth-stimulating effect of antibiotics is not entirely clear. Such chemicals may act through the selective reduction of harmful organisms in the digestive tract. Some of the earlier experiments support this concept since animals housed under less sanitary conditions showed the greatest benefit from antibiotic administration. Chicks grown under sterile conditions and those fed antibiotics have thinner intestinal linings. This could result in more complete absorption of nutrients from the small intestine and thus provide far more rapid growth. Some experimental evidence suggests that a stimulation of the pituitary gland may result from antibiotic consumption. Thus, increased growth could result from an increased synthesis of the growth hormone (page 319).

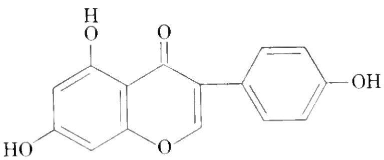
The antibiotic feed supplements on the market generally also supply a mixture of vitamins since they are prepared from fermentation broths. Vitamin B_{12} (page 345), also called animal protein factor (APF), has recently been shown to stimulate growth of poultry and swine. This vitamin and other B vitamins may play roles in the beneficial effects obtained with antibiotic supplements. Although the physiology of the stimulation is not completely understood, such antibiotic-vitamin B_{12} supplements have become very important factors in the development of feeds producing rapid and efficient growth.

Antibiotic-vitamin supplements increase the rate of gain of young calves but only to an age of about 6 months. No beneficial effects were observed in older calves or in beef and dairy cattle.

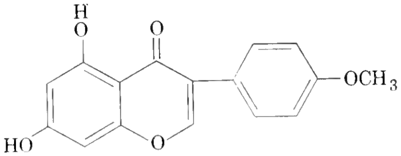
HORMONES

Recently there has been much interest in the use of synthetic sex hormones in fattening poultry and beef cattle. Diethylstilbestrol (page 323) has been used successfully for such purposes. Tablets of the hormone implanted in the head of the animal or added to the ration lead to increased gain in weight and increased feed efficiency. Such implantation or addition to the ration must be controlled, or undesirable side effects such as elevated tails, sagging loins, and mammary gland development may occur. Implantation of diethylstilbestrol in the heads of cockerels several weeks before slaughter has a feminizing effect and produces a fatter, tenderer carcass. Addition of the hormone to the ration of poultry is not nearly as effective as with beef cattle. Thyroprotein supplements have also produced added gains in beef cattle.

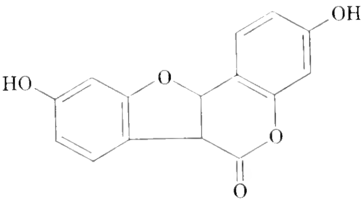
There is evidence that many plants contain compounds with estrogenic properties. A part of the beneficial effect of young pastures on milk production may be due to such hormone-like chemicals. Genistein, a 5,7,4′-trihydroxyisoflavone, has been isolated from subterranean clover in quantities of 2 mg. of the hormone per 100 g. of fresh clover. The activity per unit weight is very low compared with that of estrone, but the high content in subterranean clover makes it an important factor in sheep breeding in Australia. A similar compound, biochanin A, is found in lower quantities in red clover.



genistein



biochanin A



coumestrol

An estrogenic compound in Ladino clover has been identified (coumesterol). These compounds are dissimilar in structure to estrone (page 322). Whether they act as direct substitutes or function through the stimulation of estrone production in the animal has not been determined.

ELEMENTS

The elements essential for animal growth are discussed in Chapter 15. Most of these elements are adequately supplied in normal dietary components. Commercial mineral supplements are available, sometimes admixed with vitamins, at reasonable cost. Other preparations are often sold as cure-alls for Bang's disease (contagious abortion) and other animal diseases. There is no evidence to support such claims. The practice of feeding minerals in quantities beyond the requirements of the animal enriches only the mineral salesman.

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Digestion

17

The foods and feeds of the preceding chapter contain the vitamins and minerals discussed earlier. Along with these quantitatively minor constituents, the feeds supply carbohydrates, lipides, and proteins. Of these last most of the individual components require chemical modification before they can be utilized by animals. Thus foods are subjected by the higher animals to mechanical alteration in making the polysaccharides, lipides, and proteins more susceptible to hydrolytic attack. A combination of grinding, hydrolysis, emulsification, and absorption is termed digestion.

Man has developed aids to digestion in the form of preliminary subdivision of the natural foodstuffs, thus decreasing both the need for chewing and the period of retention of food in the stomach. In addition, foods may be cooked, thereby partly hydrolyzing starches and proteins. At the same time the latter are denatured, making many of them more quickly sensitive to the proteolytic enzymes. Finally, partial hydrolysis by acids or enzymes added to the food is sometimes practiced deliberately as in tenderizing meat by such means.

By the early part of the seventeenth century it had been suggested that digestion was analogous to fermentation. Shortly afterwards it was proposed instead that the muscular stomach ground the foods into particles small enough for direct utilization. However, this theory was invalidated by giving food in perforated, hollow-metal balls. When the experimental subject regurgitated the balls after keeping them in his stomach for a time, the food was gone. In the nineteenth century the general features of the early stages of digestion were elucidated by studies on a soldier who had a permanent opening through the abdomen into the stomach. This abnormality had resulted from a musket wound and permitted the introduction into and removal of materials from the stomach. Similar fistulas have been created in animals for studies of digestive processes.

SALIVARY DIGESTION

The first step in digestion by mammals is mastication. Teeth, tongue, and lips cooperate in breaking the food into small particles with a large surface area for subsequent attack by the digestive juices. The first of these juices with which the food comes in contact is saliva secreted into the mouth by the salivary glands and the numerous small buccal glands. There are three pairs of salivary glands, parotid, sublingual, and submaxillary glands. When food is eaten these glands are stimulated, and the flow of saliva increases. Increased salivation may also be induced by placing objects in the mouth, by the sight or thought of food, by conditioned reflexes associated with feeding, by odors and flavors, and by stimulating certain connecting nerves. Human beings secrete 1 to 1.5 liters of saliva daily and horses and cattle perhaps 40 and 50 liters, respectively. The amounts appear to vary with the nature of the food, its moisture content, and environmental and psychic factors. To some extent the composition and hence the consistency vary in a similar way.

Saliva is, of course, primarily water but contains a variety of inorganic ions like sodium, potassium, calcium, magnesium, chloride, carbonate, phosphate, and sulfate, and has a pH in the range of 6.4 to 7.0. On standing the pH tends to rise as a result of loss of carbon dioxide. In addition to the inorganics, an organic fraction consists of several enzymes, small amounts of a variety of soluble organic compounds, and mucin, a glycoprotein which forms viscous solutions and serves as a lubricant. Besides water mucin is the most abundant component in saliva.

Of the enzymes present, only ptyalin (salivary amylase) is known to be important, although even it does not occur in the salivas of the carnivores and herbivores. Ptyalin is produced in quantity by man, swine, and rodents and partially hydrolyzes starch, producing maltose and dextrins of various sizes. Since maltase also is secreted in traces, some maltose may be split to glucose but the total amount is probably quite small, partly because of the low enzyme concentration. Furthermore, the food spends such a short time in the mouth that there is opportunity only for limited hydrolysis even by the amylase. For this reason chemical changes in the mouth are probably neither extensive nor important.

After food is mixed with salivary amylase and swallowed, hydrolysis of starch continues in the stomach until the pH drops to about 4 on admixture with hydrochloric acid. While the pH is decreasing over a period depending on the quantity and nature of the material eaten, enzymatic action may continue for 15 to 30 minutes and convert 60 to 75 per cent of the starch to maltose.

GASTRIC DIGESTION

Voluntary initiation of swallowing carries the food over the epiglottis and into the upper portion of the esophagus (oesophagus, gullet). From this point the processes are involuntary, starting with a peristaltic wave induced by contraction of circular muscles in the esophagus. This wave motion is preceded by a relaxation, permitting ready movement of the swallow of food and extending to the cardiac sphincter at the junction of esophagus and stomach. The cardiac sphincter is a muscular valve keeping the stomach closed and must be relaxed for food to pass. Gastric digestion then begins in the stomach system but takes various forms depending on the species. The three different types of interest here are the single-sac stomach characteristic of most mammals, including man, the gastric system of the ruminants, and that of birds.

The Single-sac Stomach

Figure 17–1 represents a typical single-sac stomach and shows a large segment connected to the esophagus and often called the cardiac portion. At the other end the pyloric portion or pylorus is joined to the duodenum by way of the pyloric valve or sphincter. This valve controls the flow of materials from the stomach and prevents regurgitation from the intestine back into the stomach. The large central zone making up part of the cardiac portion is called the fundus and possesses cells secreting the hydrochloric acid and pepsin of gastric juice.

The empty stomach is collapsed because of contraction of muscle fibers in the walls. When the bolus of food reaches the cardiac portion of the stomach, just enough expansion occurs to accept the volume presented and to distribute the mass over the wall area. As more

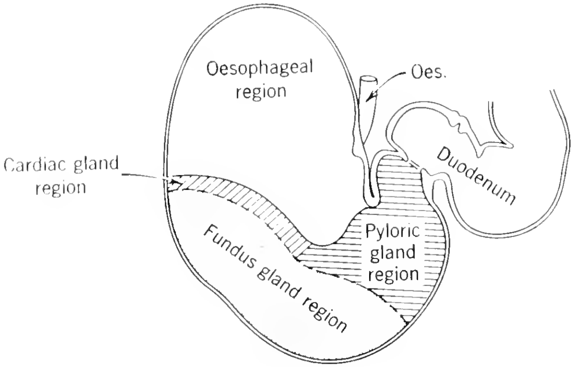

FIGURE 17-1. Diagram of the stomach of a horse. (Courtesy of W. B. Saunders Co.)

food is swallowed, the additions tend to occupy the central region since the wall is covered. Salivary action continues in this mass until gastric juice is admixed by the muscular activity of the stomach.

A continuing gentle contraction in the cardiac region presses the stomach contents toward the pylorus. In this latter region peristaltic waves slowly move the material toward the pyloric valve, which responds to a high acidity by opening momentarily and allowing a small quantity of liquid or semiliquid material to pass through. On closing, the valve temporarily reverses the general flow and aids in mixing the contents of the pylorus. Intermittent operation of this sphincter both aids in gastric digestion by prolonging the exposure time in the stomach and controls the admission of material into the intestine.

The musculature of the stomach is controlled by a highly organized nerve network providing synchronized activity. Since the network has a connection with the vagus system, strong emotion can inhibit normal stomach movements. Cramps and vomiting represent reflex responses to abnormal stimuli.

Secretion of gastric juice appears to be controlled by reflex responses to eating and to a lesser extent to the specific local chemical effects of meat components and degradation products. The rate of gastric secretion rises rapidly after a meal to a maximum in about 2 hours and then declines steadily. It seems probable that secretion occurs at low levels even for long periods without food and that the entire process is at least partially under hormone control. Although extensively studied, the mechanisms and control of gastric secretion are but poorly understood.

Normal gastric juice in man and dogs contains a variety of inorganic salts, mucins (glycoproteins), enzymes, and hydrochloric acid. The salts have no known role in gastric digestion, and the mucins are supposed to serve only as lubricants.

Pepsin is the most important enzyme of the gastric juice. It is not secreted as active pepsin but is first produced in an inactive form called pepsinogen, the precursor of pepsin. When pepsinogen comes in contact with hydrochloric acid, active pepsin is formed. Pepsin is most active in acid solutions, and it has been shown that the proteolytic activity of pepsin is not due to hydrochloric acid *per se,* for many acids, at proper concentrations, can produce satisfactory results. The optimum concentration of hydrochloric acid for the action of pepsin is in the range of pH 1.5 to 2.0, while the enzyme is inactivated above pH 6. Like many enzymes of animal origin, pepsin is very active at temperatures from 38 to 40°C.

A second gastric enzyme called rennin is active in curdling milk by catalyzing the conversion of casein to paracasein, which combines with calcium ions to yield insoluble calcium paracaseinate. Removal of calcium salts from the milk prevents coagulation. Such precipitation may aid digestion in young animals by prolonging the exposure to hydrolysis by pepsin. Both pepsin and hydrochloric acid also curdle milk though not so powerfully as rennin. Although rennin is found in the stomachs of infants and young animals, such as the calf, little, if any, rennin is secreted by adult human beings or mature animals. Hydrochloric acid and pepsin probably play the major role in gastric coagulation of milk so far as adult human beings are concerned. Rennin acts best in slightly acid solutions (pH 6.0 to 6.5), although it will coagulate casein in neutral and even slightly alkaline solutions. Commercial rennin, known as rennet, is obtained from the calf stomach and is used in the manufacture of cheese. When casein is made for industrial purposes, it is precipitated from skim milk by adding acid. At the isoelectric point (pH 4.6 to 4.8) casein precipitates, differing from calcium paracaseinate, which is of smaller molecular size.

The existence of a final gastric enzyme called lipase has been debated vigorously. Apparently it does occur in small amounts, but its role is uncertain. Perhaps this lipase hydrolyzes glycerides to a small extent, but the low pH of the stomach prevents large-scale activity.

Gastric juice is secreted by the numerous glands of the stomach lining (gastric mucosa). These glands differ somewhat in the various regions of the stomach and hence supply different relative quantities of the three fluids concerned. Mucus is secreted by the mucous cells, hydrochloric acid by the parietal cells, and pepsinogen by the chief cells. Both mucin formation and pepsinogen formation (converted to pepsin) appear to be primarily problems of protein synthesis, and little is known of these two cases. Secretion of hydrochloric acid is stimulated by histamine formed by decarboxylation of histidine. The mode of action is unknown. Likewise the mechanism of hydrochloric acid formation is still in doubt in spite of much study and the proposal of several theories. An ample supply of chloride ion is available from the blood, but the large-scale concentrating mechanism needed for the hydrogen ions has not been identified.

Gastric digestion follows the action of salivary amylase mentioned earlier and begins when secretion of gastric juice and muscular activity combine to lower pH below 4. At this acidity the salivary amylase ceases to function and pepsin becomes active. Thereafter, gastric digestion is proteolytic in nature and is catalyzed at the low pH by pepsin, which shows its maximal activity at pH 1.5, still functions, though much more slowly, at pH 3, but is inactive for practical purposes above pH 4.

It is probable that proteins must first be denatured before they are attacked by pepsin, but most proteins are readily denatured at low pH. Thus the normal environment in the stomach prepares ingested proteins for peptic hydrolysis. The hydrolysis is never complete for two reasons. Even under optimal equilibrium conditions, pepsin cannot split all the peptide bonds of any protein. Furthermore, the stomach contents are not retained long enough to reach even the equilibrium level of hydrolysis. Instead the denatured proteins are broken into large water-soluble fragments composed of several amino acid units. These polypeptides are sometimes called peptones and are digested further in the intestinal tract. Limited amounts of small peptides and free amino acids appear with the peptones but probably comprise only a small fraction of the material potentially available. Operation of the pyloric sphincter allows the digesting slurry of stomach contents to pass slowly from the stomach for the subsequent operations of intestinal digestion.

A dilemma is readily apparent in contrasting the action of pepsin on the ingested proteins with the apparent lack of hydrolysis of the stomach itself. Certainly the stomach of one animal is digestible when eaten by another individual. Therefore, a unique structure of the protein is not involved. Perhaps the cells of the living stomach somehow resist denaturation by the hydrochloric acid and thus are not susceptible to pepsin. Conceivably the gastric mucin might offer protection to the surface. In any event, whatever the unknown protective mechanism may be, it sometimes fails, and the acidity, mechanical irritation, action of pepsin, or possibly all three lead to gastric (or peptic) ulcers. In the context of this problem it is interesting to note that pepsin has some hydrolytic action on itself. Such autohydrolysis partially degrades the molecule and liberates peptides in the process. Pepsin does not attack the peptide bonds between all possible combinations of amino acids. Rather it is primarily concerned in the hydrolysis of only those bonds connecting the aromatic amino acids tyrosine, phenylalanine, and tryptophan with other amino acids, thus accounting for the incompleteness of the equilibrium hydrolysis of proteins.

Ruminants

Animals related to cattle, sheep, and goats possess a compound stomach of four sections known as (1) the rumen or paunch, (2) the reticulum or honeycomb, (3) the omasum, and (4) the abomasum or true stomach, corresponding closely to the single-sac stomach of other mammals. As shown in Figure 17–2 the first two regions are not actually separate compartments; the reticulum is really a lobe of the rumen. Furthermore the omasum is closely associated physiologically, so that rumen, reticulum, and omasum combined form a large fermentation vat. Anatomically these chambers are regarded as enlargements of the esophagus.

As a rule the ruminant masticates its food very imperfectly at the

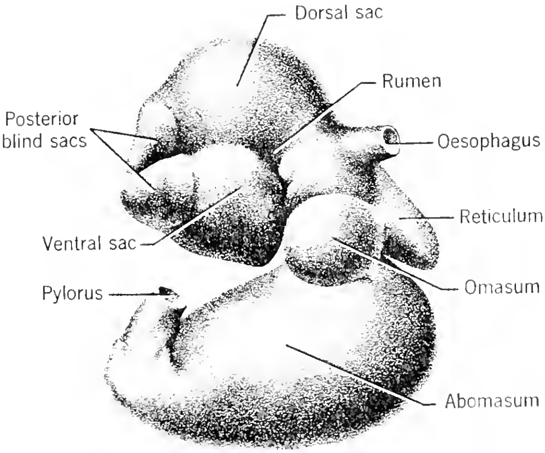

FIGURE 17-2. Ruminant stomach. Right view with rumen raised. (Courtesy of W. B. Saunders Co.)

time of eating. The liquid, soluble, and finely divided portions of the feed pass down the esophagus and through the esophageal canal directly to the true stomach. The esophageal canal, which is a continuation of the esophagus, is constructed somewhat like a rubber hose cut lengthwise so that it can be opened. So long as the contents are of a liquid nature, they pass through the tube, which remains closed by muscular contraction. However, when large masses of fibrous hay and similar materials are swallowed, the esophageal canal opens and drops the coarse material into the capacious rumen. Here the feed is brought into intimate contact with saliva and is fermented by microorganisms.

When the ruminant has completed feeding, it has the power to regurgitate and masticate this coarse material at its leisure. This process is known as "chewing the cud." From the rumen, the finely comminuted food passes to the other stomachs or compartments and eventually reaches the abomasum, where true digestion takes place. During the rumination process, methane, acids, and alcohols are formed in small amounts by fermentation. Certain members of the vitamin B complex (thiamine, riboflavin, pyridoxine, and pantothenic acid) are synthesized by the rumen organisms, which in addition partly hydrolyze cellulose and hemicellulose, making these otherwise indigestible materials available for subsequent assimilation by the animal. Moreover, the symbiotic microorganisms also convert non-protein forms of nitrogen like ammonia, urea, amino acids, purines, pyrimidines, and amides into the proteins making up the cells of their own descendants. When the organisms pass along the digestive tract after leaving the rumen, most of them die and the proteins undergo digestion by the host animal.

The microflora of the rumen consists of several species of bacteria and some protozoa. The ecological aspects of the rumen are being intensively studied in laboratory cultures and by sampling materials from the rumen itself through a fistula established surgically. Although the bacteria appear to play the major synthetic and hydrolytic roles, the protozoa are important, at least in cellulose digestion. Moreover, they ingest bacteria and convert the protein of the latter (74 per cent digestible) to forms 91 per cent digestible by the host ruminant, thereby adding to the efficiency of nitrogen utilization.

Clearly the well-being of ruminants is contingent upon the maintenance of a suitable microflora so necessary for the rumen digestion of polysaccharides. In part these microorganisms depend on a supply of minerals some of which are ingested in the food. This source is supplemented by the several ions of the saliva secreted copiously by ruminants. In part this abundance of saliva contributes liquid needed for active microbial growth and fermentation. The microorganisms secure the required energy and carbon sources by attacking the plant carbohydrates. Likewise they make all the intermediates, vitamins, and coenzymes needed for their growth.

However, the nitrogen supply sometimes limits the microbial activity which must ultimately depend upon the protein syntheses. Various forms of nitrogen have been incorporated into diets for ruminants in an effort to increase the utilization of cellulose and consequently the efficiency of grazing animals. Reduced nitrogen of almost any non-toxic type is effective, and it is now economically feasible to make urea supplements to cattle rations.

Newborn ruminants have no rumen flora and derive no benefit from cellulose and the like until a suitable flora develops. This takes from 4 to 6 weeks in the calf, probably depending on accidents of ingestion of suitable organisms. Thereafter the rumen performs its function except in abnormal cases, the most common being bloat or tympany. In this state the rumen becomes distended, perhaps with fatal results, because of excessive gas production, failure of the belching mechanism, or formation of froth in the rumen. The most common cause is associated by some unknown mechanism with extensive consumption of green legumes. Several treatments are available.

Fowl

The digestive tracts of domestic fowl differ somewhat from those of mammals and thereby warrant separate consideration. The esophagus (gullet) of chickens differs by being divided into two parts known as the first and second portions. These are separated (see Figure 17–3) by an enlargement of the esophagus known as the crop, which functions as a storage organ during the hours of feeding. It should be pointed out that no mastication takes place, since birds do not possess teeth.

From the crop the food passes through the second portion of the esophagus to the proventriculus or glandular stomach, which is a small expansion of the esophagus, measuring (in the hen) about 1½ in. in length and 1 in. in diameter. The proventriculus functions primarily as a secretory organ, furnishing gastric juice, but it differs from the true stomach of other animals in that the food does not remain long enough for gastric digestion to take place. From the proventriculus, the food, moistened with gastric juice, passes to the gizzard. This large muscular organ, with the aid of grit, grinds the food material to a fine state of division. During the grinding

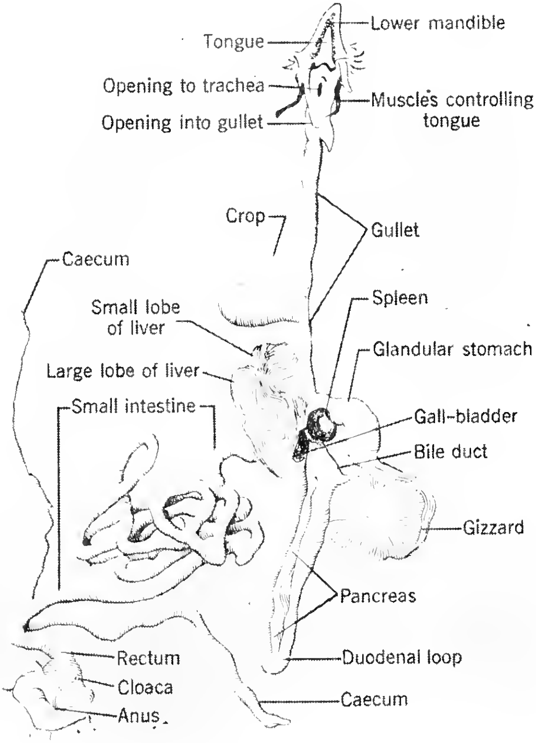

FIGURE 17-3. Showing various portions of the digestive tract of the domestic fowl. (After Lippincott.)

period considerable gastric digestion takes place. The finely ground contents are then passed on to the small intestine.

INTESTINAL DIGESTION

The pyloric sphincter opens from time to time in response to the semifluid stomach contents of low pH presented by the peristaltic waves of the pylorus. As soon as sufficient of the material called chyme passes through the sphincter to lower the pH at the intestinal wall, the pyloric sphincter closes. It thus admits chyme in intermittent small portions and prevents overloading of the intestine and an excessive acidity. Meanwhile gastric digestion continues and the stomach acts as a storage organ, allowing the animal to eat occasional large meals instead of limited but frequent ones. When solid masses of food are presented by peristaltic action, the pyloric sphincter does not open. Nor does the valve operate normally when insufficient hydrochloric acid has been secreted to lower the pH. These properties insure a suitable consistency and a reasonable extent of gastric digestion.

Chyme enters the duodenum, a portion of the small intestine about 11 in. long in man, and comes progressively under the influence of three digestive juices. Pancreatic juice and bile are secreted from separate ducts but into the same region of the intestine, so that their activities are interdependent. Moreover, intestinal juice also acts conjointly though this fluid is secreted by several types of small glands located throughout the intestinal mucosa. For convenience the compositions and functions of the three digestive juices are considered separately even though they intermingle and act together.

Pancreatic Juice

The pancreas is a tubular gland lying along the duodenum and secreting intermittently into the intestine. Its activity is stimulated by contact of the acid chyme with the walls of the duodenum, which in turn produces hormonal material initiating pancreatic secretion. The basis for this statement lies in the fact that maceration of duodenal tissue with hydrochloric acid produces an extract which stimulates the flow of pancreatic juice when injected into the blood stream of another animal. If intestinal extract that has not been treated with HCl is injected, no pancreatic stimulation occurs. Furthermore, acid extracts of other organs have no effect.

Proof of the transfer of secretin by the blood was shown by a most ingenious experiment. The blood streams of two dogs were connected in such a manner that the blood supplies intermingled extensively. Tubes were placed in the pancreatic ducts of both animals, and a dilute acid solution was introduced into the duodenum of one of the dogs. The acid caused pancreatic secretion simultaneously in both dogs, proving that secretion of secretin took place in one dog and was transferred by the blood to the other animal.

More recently, participation of at least two hormones has been demonstrated. Of these secretin has been purified and shown to be a complex polypeptide. It specifically stimulates the production of fluid containing sodium bicarbonate. A second hormone, pancreozymin, induces enzyme production, and the two hormones, perhaps with additional unknown stimuli, result in the secretion of normal pancreatic juice. The two known hormones come solely from the duodenum, other portions of the intestine being incapable of stimulating the pancreas.

The duodenum supplies yet another type of activator called enterokinase, though there is now some doubt that the material is an enzyme. Enterokinase is secreted into the chyme and as a result comes in contact with pancreatic juice. The latter fluid contains certain enzyme precursors whose conversion to active enzymes is initiated by the enterokinase. For example, enterokinase changes trypsinogen to trypsin, the active form. Once some trypsin appears it catalyzes its own production, the phenomenon of autocatalysis.

Pancreatic juice supplies enough sodium bicarbonate to neutralize the acid of chyme and bring the intestinal contents to pH 8 to 9 for the effective action of intestinal and pancreatic enzymes. Pancreatic juice also contains a number of enzymes, several of them very extensively studied, and taken together they contribute to the digestion of proteins, fats, and carbohydrates.

Trypsinogen and chymotrypsinogen are converted from the zymogens to the corresponding active forms. It now seems that there is a family of chymotrypsins, all similarly active but of slightly different sizes, which result from the removal of different peptide fragments during the activation steps. Both trypsin and chymotrypsin are proteolytic enzymes and attack different kinds of peptide bonds from those attacked by pepsin. Thus the proteolysis is carried farther, and any proteins previously escaping gastric digestion are partly degraded by trypsin and chymotrypsin. Hydrolysis of proteins to amino acids is continued by carboxypeptidase, activated from procarboxypeptidase by trypsin. Carboxypeptidase has little action on proteins but attacks polypeptides by removing amino acids one at a time from the free carboxyl end of the chain. Since some types of peptide bonds are not rapidly hydrolyzed, additional enzymes are needed. These occur in intestinal juice, and they are discussed in that connection on page 437.

Pancreatic amylase (perhaps two separate enzymes) converts starch and the soluble degradation products from the stomach into maltose. As maltose is formed, maltase splits it into glucose. Likewise lactose, so important in the diets of young animals, is hydrolyzed by pancreatic lactase. Again these enzymes are supplemented by similar catalysts in intestinal juice. A lipase (steapsin) also occurs in pancreatic juice and induces hydrolysis of neutral fats. Pancreatic lipase appears to be the major enzyme of fat digestion.

Bile

Among the many important functions of the liver, one is the formation of bile. Liver secretes bile continuously, although the amount fluctuates somewhat with the feeding schedule and is apparently subject to some sort of partial control. Bile flows from the liver along a duct and through another valve-like sphincter which controls passage into the intestine. Passage of acidic chyme through the pyloric sphincter into the duodenum causes the sphincter of the bile duct to open. When the bile sphincter is closed, the continuing flow of bile is stored in the adjoining gall bladder, which is provided with muscle fibers and imposes a gentle pressure somewhat in the manner of the stomach. This pressure expels bile from the gall bladder on opening of the sphincter.

The gall bladder is primarily a storage organ, but it does modify in two ways the bile as originally secreted by the liver. First, the bladder reduces the water content and thus relatively increases the concentrations of other materials. Second, the gall bladder secretes additional supplies of some of the bile components. Although the storage, concentrating, and secretory functions are important, they are not essential since surgical removal of the gall bladder still allows the animal to digest comparatively normal diets. Such operations are, of course, fairly common in human beings, occasioned mainly by the formation of "stones" in the gall bladder. These stones originate by precipitation of cholesterol from the concentrated bile stored in the bladder. Although such precipitation is commonplace, other components normally prevent aggregation to an extent causing blockage of the duct.

The composition of bile is summarized in Table 17–1. Two columns are given to permit comparison of bile directly from the liver with that from the gall bladder. With a change in water content leading to an increase in the total solids from 3 per cent to 14 per cent, an increase by a factor of about 4.5 should occur for the individual

TABLE 17–1. Composition of Human Bile

	Percentage Composition	
Component	From Liver	From Gall Bladder
Water	97.1	86.0
Bile salts	1.6	8.2
Inorganic salts	0.72	0.78
Mucin and pigments	0.49	2.3
Cholesterol	0.12	2.2
Fat and lecithin	0.06	0.66

components unless they too are altered in the bladder. Lipides, including cholesterol, fats, and lecithins, are supplemented in the gall bladder while salts are withdrawn, maintaining a rather constant salt concentration.

No special function is known for the mucin and inorganic salts of bile. The bile pigments are important as excretory forms of nitrogen and are discussed in connection with nitrogen metabolism on page 471. These pigments are excreted in feces and represent waste products of liver activity. Roles are not known for the fats and lecithins of bile although they should be subject to the same digestive processes as are analogous ingested materials. Cholesterol (see page 93) is partly derived from ingested animal materials, at least in herbivores, is partly formed from plant sterols, and is partly synthesized in the liver from acetate by a complex reaction pathway. Again for cholesterol, the function in bile is not known. Much of it is excreted in the feces, and in cases of atherosclerosis (hardening of the arteries) cholesterol is deposited in artery walls. As a result there is much interest in all aspects of the physiology of cholesterol.

Bile salts form the most important biliary component, making the major contribution to intestinal digestion. This group of organic salts effectively emulsifies dietary lipides of all kinds. As a result of this powerful surface activity, ingested fats subdivide into minute droplets whose high surface-area-to-volume ratio makes them much more efficiently hydrolyzed by lipase. Furthermore, the fats coating particles of carbohydrate and protein are removed and digestion of these food components is facilitated. Thus bile makes an important contribution to many aspects of intestinal digestion though it does not directly catalyze hydrolyses. When the flow of bile is blocked for any reason, digestion is impaired in general, and most of the fat is excreted and lost. Disease states of this type, which are termed jaundice, most often result from physical obstruction of the bile duct or from liver failure due to poisoning or infection as in infectious hepatitis. The absence or shortage of bile during jaundice does not permit proper emulsification of fats, and much fat cannot be digested and is lost by excretion.

The compounds termed bile salts are structurally related to and may be derived in part from cholesterol. They are amides of complex acids with glycine or taurine, $^{+}H_3NCH_2CH_2SO_3^{-}$, which is formed from cystine and cysteine. In human bile four different acids occur, the most abundant being cholic acid (see page 94). At the pH of bile and intestinal contents, the bile salts exist as anions associated with inorganic cations.

Intestinal Juice

This fluid contains the enterokinase mentioned earlier as the activator of trypsinogen. In addition several hydrolytic enzymes occur in the secretion, including the material formerly called erepsin. It is now known that the erepsin consists of the pancreatic enzyme carboxypeptidase and the intestinal enzymes aminopeptidase, dipeptidase, aminotripeptidase, and prolidase. Of these, carboxypeptidase has been discussed (page 434). Aminopeptidase likewise attacks polypeptides one step at a time but begins at the end carrying the free α-amino group. Aminotripeptidase has a similar activity but is restricted to tripeptides. Dipeptidase hydrolyzes dipeptides, and prolidase the dipeptides of proline, as suggested by the respective names. Most of these enzymes, classed as peptidases, require divalent metals for catalytic activity. Carboxypeptidase specifically depends on zinc and prolidase on manganous ion. Aminopeptidase and dipeptidase can function with any of a number of related ions. Taken together, all these enzyme systems cooperate to hydrolyze proteins almost completely into free amino acids.

Along with the peptidases, intestinal mucosa secretes carbohydrases acting on the disaccharides sucrose, maltose, and lactose. These enzymes, sucrase (or invertase), maltase, and lactase, yield readily utilized monosaccharides from the otherwise somewhat inert parent sugars.

Additional digestion is produced by enzymatic cleavage of nucleic acids. Nucleases catalyze the first stages to nucleotides, attacked in turn by nucleotidases, which are then split by nucleosidases and phosphatases to the purine and pyrimidine bases, pentoses, and phosphate.

Absorption

The intestinal tract is composed of three major segments. The first of these, the small intestine, is tubular, beginning at the stomach and extending to the caecum. In turn the small intestine is divided into three parts called the duodenum, jejunum, and ileum. The overall length of the small intestine varies with the species and the individual, averaging 9 times the body length in horses, 16 times in sheep and cattle, and 11 times in swine. The ileocaecal valve separates the small intestine from the caecum and controls the flow of materials.

The caecum is an enlargement of the alimentary canal and serves as a storage organ for further digestion and fermentation. No digestive juices are secreted here, but the organ is of great importance in spite of this fact. In a way it might be compared with the rumen or paunch of ruminants, although it differs from the rumen in that digestive changes take place in the caecum, owing to the presence of the enzymes originating in the pancreas and small intestine. In a general way, the size of the caecum is inversely proportional to that of the stomach. In the horse the caecum is very large and holds about 16 per cent of the total contents of the digestive tract. This organ allows the food to remain for long periods of time in contact with the digestive juices from the small intestine, and, as a result of bacterial action, the horse is able to digest and utilize crude fiber to a very appreciable extent. The caecum in ruminants is relatively small, with a capacity of about 3 per cent of the tract. That of hogs is not much higher, being about 5 per cent.

Located near the intestinal inlet to the caecum is another tube, somewhat larger, called the colon, forming one end of the large intestine. An enlarged section near the terminus or anus is called the rectum. In the horse the large intestine is also highly developed and may constitute as much as 45 per cent of the total capacity of the digestive tract; like the caecum, it serves as a storage organ in which the hydrolysis of difficultly digestible foods continues. In the domestic fowl two caeca connect with the intestine at the same point, forming a Y-shaped structure (see Figure 17–3).

Digestion products are not assimilated to appreciable extents until the small intestine is reached. Some exceptions include alcohol, salts if the food is very high in minerals, and components of certain condiments like pepper and mustard. The small intestine is lined with folds of tissue containing a multitude of tiny projections known as villi. Although the duodenum is the section most densely populated with villi, the jejunum and ileum contain many, and since the last two sections are very long they probably make the major contributions to absorption.

The villi are supplied with two circulatory systems, a blood system consisting of arterial and venous networks, and a lacteal or single lymphatic capillary which communicates with the lymphatic system. From the villi, absorbed nutrients are carried via the mesenteric veins to the portal vein and thence to the liver and body tissues. A major portion of the fats, however, is absorbed in the lacteals and reaches the tissues via the lymphatic system.

The role of bile in fat digestion has already been mentioned. Glycerol, a normal product of fat hydrolysis, is water soluble and readily absorbed. Fatty acids, however, are not water soluble and cannot be absorbed as free acids. Consequently they form soluble complexes with bile salts, in which form they diffuse through the intestinal membrane. The bile salt complexes dissociate as soon as they are absorbed, and tissue lipases resynthesize glycerides from the absorbed glycerol and the free fatty acids released from the bile salt-fatty acid complexes. The bile salts eventually return to the liver, where they again become normal bile constituents.

Although the fatty acid-bile salt complex explanation for fat absorption is generally accepted, some unhydrolyzed fat is definitely absorbed in highly emulsified form, with globules so small that they can pass through the intestinal mucosa. In fact, much if not most of the fat absorbed during digestion is absorbed as finely emulsified unhydrolyzed glycerides. As suggested in a previous paragraph, a large proportion of the absorbed or resynthesized fat is shunted around the liver by being carried to the tissue via the lymphatic system. This fat-rich lymph is carried by the thoracic duct, where it is poured into the blood stream at the junction of the jugular and subclavian veins. This creamy lymph is called chyle.

Carbohydrates are absorbed by the capillaries of the villi in the form of glucose, fructose, and galactose, and these simple sugars are carried by the blood stream to the liver, where they are deposited as glycogen (animal starch), or they are taken to the tissues, where they are utilized or deposited as glycogen. Some disaccharide absorption may take place, but it is considered of minor importance. Although glucose, fructose, and galactose may be converted in the liver and muscles to glycogen, it should be noted that tissue glycogen undergoes hydrolysis only to glucose.

Lactose, because of its relatively low solubility, is not utilized so efficiently as other sugars. As a result an appreciable amount of lactose finds its way to the lower intestine, where it often plays an important role in increasing the lactic acid-forming microorganisms of the intestinal flora. The metabolic products of these organisms tend to discourage the growth of putrefactive species in the intestine.

It is now believed that proteins are primarily absorbed in the form of amino acids. While it is possible that some peptides or even polypeptides may be absorbed, peptidases in the cells of the intestinal mucosa effectively convert these intermediates into amino acids. Since the origin of blood peptides is unknown, perhaps some of these materials occur by escaping hydrolysis. Such a possibility is reinforced by the connection of ingested proteins with food allergies. Either proteins themselves or large fragments are thought to be absorbed on occasion, leading to allergic responses in sensitive individuals.

During processing in the small intestine, peristaltic waves move the contents along until the large intestine is reached. The digestive changes that take place here are not of great importance. The mucous membrane of the large intestine contains gland cells, but the secretion is largely mucous and without digestive enzymes. The chemical changes that do take place here are due largely to bacteria, but enzymes are carried through from the small intestine, and some hydrolytic changes may occur. Like the secretions in the small intestine, those in the large intestine are usually alkaline. Putrefaction results in the production of proteoses, peptones, amino acids, and other decomposition products such as indole, skatole, and ammonia. In addition to the putrefactive changes, polysaccharides, such as cellulose, are acted upon by fermentive organisms, leaving behind lactic, butyric, and acetic acids, alcohols, and gases such as methane, carbon dioxide, and hydrogen.

Water is absorbed in the large intestine, and the undigested mass gradually takes on the characteristics of feces. Feces are composed mainly of undigested food materials such as cellulose, keratin, and other proteins, bacteria, and fats plus any digested nutrients that have escaped absorption.

It has been estimated that nearly one-third of the solid matter of normal feces consists of bacteria. The bacterial flora of the intestine is responsible for a variety of substances. The production of acids and ethyl alcohol is beneficial because the body can absorb and utilize these compounds. Moreover, under proper conditions, the microorganisms synthesize appreciable amounts of such vitamins as thiamine, riboflavin, folic acid, and vitamin K. Biosynthesis of vitamins has been demonstrated in the paunch of ruminants and in the digestive tracts of rats and human beings.

DETOXICATION

The end products of carbohydrate breakdown in the intestine are usually harmless, whereas certain products resulting from protein breakdown are distinctly toxic. Many of the ill effects due to constipation are tentatively attributed to the absorption of these toxic products. It is well known that intestinal putrefaction produces products that are potentially poisonous and that the body possesses ways and means of protecting itself against these toxic materials. These defenses are known as detoxication mechanisms.

The toxic products produced consist almost entirely of substances formed from amino acids by decarboxylation to amines. Known examples are listed on page 441. Histamine is also formed in the body

Amino Acid	Amine Formed
Arginine	Putrescine (tetramethylenediamine)
Lysine	Cadaverine (pentamethylenediamine)
Phenylalanine	Phenylethylamine
Tyrosine	Tyramine (hydroxyphenylethylamine)
Tryptophan	Indolethylamine
Histidine	Histamine (imidazolylethylamine)

tissues and stimulates gastric acidity. In anaphylactic shock, histamine is found in the blood in dangerously large amounts. The body attempts to protect itself against histamine toxicity by means of an enzyme, histaminase, found in the intestine.

The liver is important in detoxication and destroys many toxic compounds. The kidneys inactivate still others. Benzoic acid from intestinal putrefaction is detoxicated by condensing with glycine to form the harmless excretory product, hippuric acid. Likewise, toxic phenols are excreted as harmless esters of sulfuric acid or glucuronic acid. In other words, the body has a number of protective mechanisms for detoxicating harmful products formed in or taken into the body, including reductions, hydrolyses, oxidations, and esterifications.

$$\underset{\text{benzoic acid}}{C_6H_5 \cdot COOH} + \underset{\text{glycine}}{{}^{+}H_3N \cdot CH_2 \cdot COO^-} \xrightarrow{\text{hippuricase}} \underset{\text{hippuric acid}}{C_6H_5 \cdot CO \cdot NH \cdot CH_2 \cdot COOH}$$

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Carbohydrate metabolism

18

Much information directly pertinent to the metabolism of carbohydrates by animals has been already presented. Chapter 7, Energy Transfers and Biological Oxidations, and Chapter 9, Plant Metabolism, summarize much that is important here. Since many of the metabolic reactions take place in cells of all kinds, work done with plants and microorganisms has direct application to animals. The occasional exceptions make it necessary to repeat experiments with several different species but seldom render it impossible to write schematic summaries covering large groups of metabolic reactions for many living forms.

Generally speaking, carbohydrate metabolism in animals begins with the ingestion of food. The first step thus becomes digestion, the subject of Chapter 17. Digestion involves two overall processes. First, the large molecules are partially degraded by hydrolytic enzymes, and, second, the small molecules and the intermediate hydrolytic fragments are absorbed by the intestinal tract. The details of these processes have been covered in the preceding chapter and need not be presented again here.

After digestion has occurred, the metabolites thus provided are distributed by the circulatory system to the organs and tissues utilizing them. Some of the compounds concerned are transported as simple solutes in solution, others in complexes with proteins. Often the supply of metabolites from digestion and the demands for energy, growth, and tissue repair differ. Therefore, excessive supplies must be stored temporarily until a need arises.

This arrangement is so common it arouses little curiosity, for it must occur with each meal, since higher animals all eat more or less intermittently. Yet the control of storage processes is complex, partly because the osmotic concentrations of blood, interstitial fluid, and cells must be kept fairly constant. During and immediately after a meal, the concentration of small metabolites rises in the blood. This increase is controlled to prevent osmotic damage by converting the material not immediately needed into forms having little or no effect on the osmotic concentration. Obviously this process must be reversible to meet demands arising before the next feeding period.

Osmotic concentration depends, of course, only on the numbers of particles and not on their sizes. Therefore, avoidance of excessive osmotic concentration becomes simply a matter of polymerizing the small molecules into very large ones or converting them into insoluble substances. Both schemes are employed, leading to polysaccharides and lipides, respectively.

Animals convert the excess of digested carbohydrates into insoluble lipides by the reactions of pages 457 to 460. This energy reserve then becomes available for mobilization whenever the food supply does not meet the energy requirement. Since the lipide is stored in special depots and is not very soluble, fat is not rapidly available and is not adequate for the demands raised by sudden bursts of activity.

To provide for major physical exertion between eating periods, a more readily available energy reserve is needed. The polysaccharide called animal starch or glycogen fulfills this role. Glycogen is stored in cells throughout the animal body, but its great molecular size allows the accumulation of quite useful weights without much effect on the osmotic concentrations of intracellular fluid. Since fat is more highly reduced than carbohydrate, it is a more condensed form in which to store energy. Thus fat serves as the major supply of stored energy while glycogen acts as the immediate reserve, sufficing for some hours without food intake.

GLYCOLYSIS AND GLYCOGENESIS

Glycogen in Liver

Although, as mentioned above, glycogen is known to occur throughout an animal, even in certain areas of the depot fat, it is most abundant in skeletal muscle and liver. Most of the biochemistry of glycogen has been based on work with these two tissues.

In mammalian species the liver is normally about 2 to 8 per cent glycogen, undergoing a rapid turnover. A starvation of 24 hours almost completely depletes the liver of glycogen, but restoration is rapid as soon as a suitable diet is ingested. Even with average food intakes the glycogen is continually broken down and replaced, although the day-to-day levels change little. A system of this kind is called a dynamic equilibrium or steady state. In rats on normal rations, glycogen has a half-life of about 1 day, meaning that half the initial glycogen is utilized daily and replaced from glycogenic materials of the diet.

Glycogenic substances are those potentially leading to the formation of glycogen and include all materials that directly and indirectly yield glucose-1-phosphate. Once formed, this last compound is converted to liver glycogen by the enzymatic reactions summarized on page 220. In studying glycogenesis, the compounds to be tested are fed to rats previously starved until their livers are depleted of glycogen. If a compound can be converted to glycogen, the latter is found upon analysis of the liver. Thus it is possible to discover the compounds that can lead to glycogen after starvation.

However, not all these compounds will necessarily yield glycogen when they are added to otherwise normal diets. Glycogen will be formed, of course, but since the level is relatively constant and may be maintained from other dietary components, a simple glycogen analysis cannot identify the source. For such studies, radioactive compounds must be used, glycogen isolated, and its radioactivity examined. Much less information of this type is available, but a number of compounds probably contribute to the glycogen supply even under normal dietary conditions.

The glycogen content of liver varies in any given individual with the composition and quantity of the diet, physical activity, lack of oxygen, acidosis (abnormally low pH of body fluids), and hormones. Administration of epinephrine, an adrenal secretion, stimulates the conversion of glycogen to glucose and rapidly depletes the liver. Other hormones also alter glycogen levels, some promoting increases, others decreases. Insulin with its pronounced effects on the concentration of glucose in blood might be expected to affect liver glycogen also. However, there is not a marked influence by insulin, and in some animal species none at all. The situation is often quite different in muscle from that in liver.

Glycogen is digested by hydrolytic reactions in the intestinal tract just as other polysaccharides are. Inside cells the endogenous glycogen is degraded by phosphorolytic processes instead, and breakdown takes place by the reversal of the synthetic steps (page 220). Thus glucose-1-phosphate is the primary product rather than glucose. In this way free energy is conserved and use of ATP avoided in bringing the glucose into the metabolic pathways providing energy and intermediates. Since glycogen is a branched molecule, it will be recalled (page 220) that enzymatic provision is necessary in the formation and removal of the branches. Glucose-1-phosphate not used in the liver cells themselves is apparently converted to glucose-6-phosphate and hydrolyzed by a phosphatase. The resulting glucose is taken by the blood to other cells needing it.

Glycogen in Muscle

Muscle contains only about 0.5 to 1 per cent glycogen, much less than is usual in liver. Nevertheless, the total quantity of glycogen stored in muscle is large because this tissue is abundant (25 to 30 kg. in human beings). Starvation does not deplete the glycogen of muscle so rapidly and completely as it does that of liver. However, the severe stresses of muscle convulsions can reduce muscle glycogen to zero.

When the hormone epinephrine is administered to well-fed animals, the liver rapidly loses its glycogen, as mentioned above. However, when a fasting animal is given epinephrine, glycogen is lost from the muscle and appears in the liver. The sequence of processes goes somewhat as follows:

The hormone activates phosphorylase, which converts glycogen to glucose-1-phosphate, and phosphoglucomutase converts this intermediate to glucose-6-phosphate. Muscle cells apparently do not contain a phosphatase for conversion of either glucose phosphate to glucose. Hence, glucose-6-phosphate is carried down the glycolytic pathway (page 168) to pyruvate and lactate. These intermediates diffuse into the blood stream and are transported to the liver, where they are converted to glycogen which is retained at least temporarily.

Although muscle cannot convert glycogen to glucose, it can use glucose in making glycogen and takes this sugar from the blood for such a purpose. The reactions are the same as those of the liver. Rare inherited diseases impair glycogen utilization in either the liver or muscle, leading to excessive accumulation of the carbohydrate in the tissue affected and reducing its availability as an energy reserve.

Glucose Levels in Blood

Most animal cells depend on glucose from the blood for the energy they need for synthesis, repair, and mechanical activity. Once ingested food has been digested, the reserves of glycogen are mobilized to supply needed glucose to the circulatory system. A continual supply is essential since the central nervous system can store very little glycogen and thus is dependent on the glucose of the extracellular fluid. Apparently glucose is one of the few energy metabolites that can pass from the blood into the brain cells. A sharp drop in blood glucose for any reason causes an immediate response leading to coma and death. Therefore, control of glucose levels in blood is essential in all the higher animals. Several materials participate in this control system.

In fasting animals blood glucose drops to characteristic levels and changes but little more when the fasting is prolonged unless the reserves become depleted and death is near. For man this plateau value is in the neighborhood of 70 to 90 mg. of glucose per 100 ml. of blood. The range indicated reflects differences between apparently normal individuals.

After eating, the glucose content of blood increases somewhat, the exact amount depending to some extent on the individual but more so on the nature of the diet. A ration high in glucose, fructose, or sucrose, for example, increases blood glucose until absorption from the food is complete. As long as the level remains below a threshold value (about 160 mg. of glucose per 100 ml. of blood for man), the kidneys prevent loss of more than traces of glucose in the urine. Although glucose is readily dialyzed from the blood, it is almost completely reabsorbed and returned to the blood stream. If the blood levels exceed the characteristic threshold, the kidney is not completely effective and glucose appears in the urine. In some pathological states and with some drugs, the threshold is lowered and glucose is excreted when the blood level is within normal limits. Other abnormalities cause the blood levels to rise above the threshold, and glucose is lost under these conditions also.

Normal individuals can tolerate the ingestion of 1 g. of glucose per kg. of body weight without the appearance of reducing sugar in the urine. Blood levels rise sharply from the fasting value to 150 mg. per ml. for most persons, and then begin to decline sharply. As the level rises in the blood, the muscle and liver cells assimilate increasing quantities of the sugar and convert it into glycogen. A part of this accelerated utilization is correlated with increased activity in the pancreas, which secretes the hormone insulin at an additional rate when the glucose content of the blood rises.

Insulin is one of the major factors in the control of blood glucose. This hormone is a protein whose structure has been much studied and is fairly well established. Normally synthesized by special cells, insulin promotes the conversion of blood glucose into cellular glucose-6-phosphate. Either insulin increases the penetration of glucose into the cell or it increases the activity of the phosphorylating enzyme glucokinase or perhaps both. Some investigators believe that a large part of the glucokinase reverts to an inactive form in the absence of insulin but that this process is reversed when insulin is administered.

A lowered utilization of glucose due to a deficiency of insulin is called diabetes. The disease is characterized by high levels of blood glucose after ingestion of sugars and by urinary excretion of glucose (called glucosuria). Weight loss, impaired muscular activity, poor healing of wounds, and nervous disorders are other symptoms and increase in severity with the degree of metabolic disturbance. The rather mild forms common in older people can usually be controlled with diets low in soluble sugars. More severe cases, especially those in children, require the regular administration of insulin since the hormonal deficiency is much greater in such people. Some progress with other drugs has been reported in those individuals still synthesizing a little insulin. Apparently it is possible to stimulate formation of the hormone if the specialized cells have not completely atrophied. Formerly life expectancy was very low for diabetic children and other individuals with severe cases. Now availability of insulin from cattle, sheep, and swine, together with increased dietary knowledge has assured diabetics of reasonably long and productive lives.

Administration of an excess of insulin or hyperactivity of the secreting cells dangerously reduces blood glucose and may lead to the comatose condition called insulin shock, an ever-present hazard in the treatment of severe diabetes. In normal animals the hypophyseal hormone antagonizes the effect of insulin, tending to increase the level of blood glucose. Epinephrine and the hyperglycemic-glycogenolytic factor both operate with a similar result by promoting the conversion of glycogen to glucose.

Thyroxin, the special amino acid hormone of the thyroid gland, catalyzes the general level of metabolism and indirectly the utilization of glucose. Yet excessive activity of the thyroid can, in fact, somehow simulate mild diabetes. Steroids from the adrenal cortex also indirectly affect blood glucose and tend to elevate the levels. Thus the level of glucose in the blood depends on the complex interplay of diet, activity, and a number of hormones.

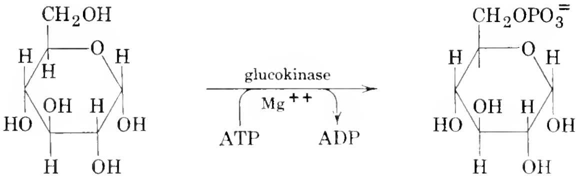
METABOLISM OF OTHER CARBOHYDRATES

Ingested polysaccharides may be either digested or excreted, depending on the animal species and the nature of the carbohydrate. Glycogen and plant starches are hydrolyzed by higher animals in their digestive tracts. Hydrolysis proceeds to the glucose stage, and this substance is then distributed by the circulatory system and used directly or converted to glycogen and fat for storage.

Many mammals cannot digest most other polysaccharides, including the pentosans, inulin, cellulose, hemicellulose. Apparently there has been no evolutionary development in higher animals providing for the direct hydrolysis of such widespread plant materials by animal enzymes. However, some species, particularly the ruminants, possess gastrointestinal systems especially adapted for bacterial hydrolysis of polysaccharides. The hydrolytic products are then metabolized by the routes available for monosaccharides. This adaptation in some of the herbivores makes available a large part of the ingested cellulose and at least part of the other polysaccharide material. The general operation of ruminant digestion has been discussed beginning on page 429.

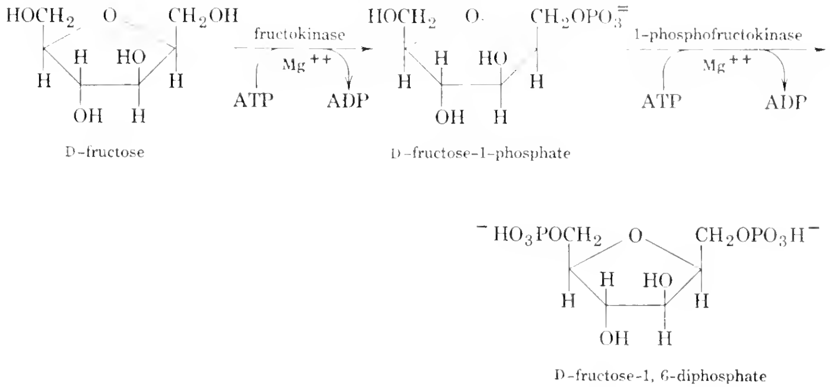
Of the disaccharides, sucrose is a major plant sugar, and its utilization would be advantageous to plant-eating animals. Enzymes variously called sucrases, invertases, or saccharases occur in mammalian intestinal tracts and split sucrose into glucose and fructose. The sugar maltose, formed during the digestion of starch and glycogen, is cleaved in the intestinal tract by maltases to glucose. A third disaccharide, lactose, is of particular importance to young mammals since it is the principal carbohydrate of milk. Once again the hydrolysis is intestinal and catalyzed by enzymes, in this case called lactases.

The three hexoses, glucose, fructose, and galactose, thus made available in quantity plus the less common mannose are phosphorylated by means of ATP, Mg^{++}, and specific kinases according to the mechanism:



α-D-glucose D-glucose-6-phospha

This same enzyme also catalyzes the conversion of D-mannose to D-mannose-6-phosphate. In the case of D-galactose the enzyme is galactokinase and leads to D-galactose-1-phosphate rather than the 6 isomer. The situation is a little more complex for D-fructose with two known pathways. First, D-fructose is phosphorylated as above for D-glucose



but with the enzyme hexokinase, and D-fructose-6-phosphate is formed, at least in some tissues. On the other hand, when D-fructose-1-phosphate is formed first, another route is utilized.

The hexosephosphates thus formed are all in equilibrium with each other by way of the reactions of page 218. Subsequent metabolism follows either the glycolytic route (page 168) through the tricarboxylic acid cycle (page 171) or the pentosephosphate pathway (page 223). It is not yet possible to decide which system, if either, is preferred and processes the major fraction of the material. Energy for synthetic or mechanical purposes becomes available at all points that lead to the formation of DPNH, TPNH, ATP, and acyl coenzyme A.

After digestion has taken place, glucose is the sugar that is widely distributed in appreciable concentration throughout the animal body. Lactose, of course, is abundant in milk but not elsewhere, and traces of various other sugars have been detected in particular organs or tissues. Fructose seems to play one special role, for this sugar occurs in seminal fluids and serves as the major energy source for the highly motile and rapidly metabolizing spermatozoa. For some reason fructose takes the places of glucose in the nutrition of the sperm cells of higher animals.

Pentoses, ingested as such or in nucleic acids or formed by the bacterial hydrolysis of pentosans, can probably be metabolized to a considerable extent by animals. Specific kinases catalyze phosphorylation of free pentoses, and the pentosephosphates may then break down by the system of page 223 involving a 3-phosphoglyceraldehyde. This last compound can go via pyruvate (page 154) to acetyl coenzyme A.

As usually written (page 171), the tricarboxylic acid cycle provides for the complete conversion of the carbon of pyruvate to carbon dioxide. However, operation in this way does not continuously sup-

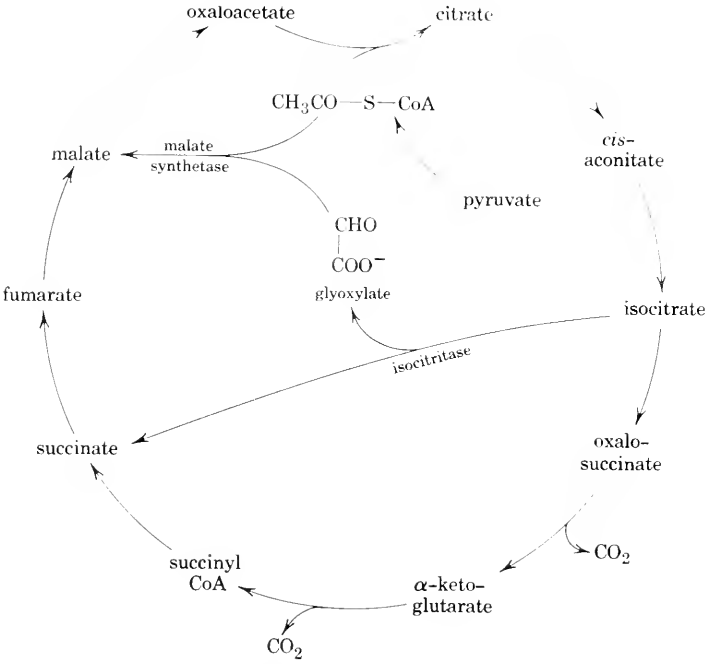


FIGURE 18-1. The glyoxylate by-pass mechanism of the tricarboxylic acid cycle. A more complete presentation of the cycle itself is given in Figure 7-2, page 171.

ply intermediates for biosynthetic purposes. If α-ketoglutarate, for example, is withdrawn in making glutamic acid, the original supply of oxaloacetate is not replaced and the cycle should stop. This problem of using the Krebs cycle for both complete oxidation and the supply of intermediates has recently been resolved as follows.

When any of the compounds of the cycle are withdrawn, isocitrate is diverted through the glyoxalate by-pass shown in Figure 18–1. The two key enzymes yield succinate and glyoxylate first, and the latter then is converted to malate by addition of carbon from acetyl CoA. Oxaloacetate is regenerated by oxidation. The net effect, the formation of a new molecule of a four-carbon acid by utilizing two molecules of acetyl CoA, becomes possible because the steps eliminating carbon dioxide are by-passed. This new molecule may be used in other reaction systems, or it may be enlarged by reaction with still another acetyl CoA to form citrate, which may proceed in turn to α-ketoglutarate by decarboxylation. In this way the tricarboxylic acid cycle continuously supplies α-ketoglutarate for glutamic acid, fumarate and oxaloacetate for aspartic acid.

METABOLIC INTERRELATIONSHIPS BETWEEN CARBOHYDRATE, FAT, AND PROTEIN

Connections between the metabolic systems for carbohydrates, fats, and proteins have been mentioned in a number of earlier sections. These linkages involve aspartic and glutamic acids, alanine, glycerol, and acetyl coenzyme A. They are represented schematically in Figure 18–2. As shown here, the arrows indicate the direction of catabolism, the breakdown of foods, and intermediates for the energy they contain. Anabolism is the opposite aspect of metabolism and refers to the synthesis of the cellular components used for other purposes than solely as sources of energy.

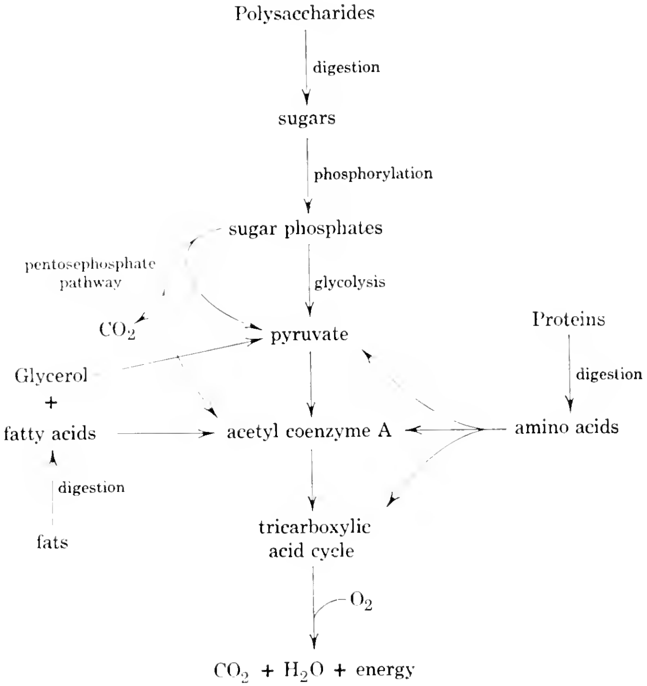


FIGURE 18-2. The relationships between the metabolisms of carbohydrates, proteins, and lipides. In most cases each arrow represents a complex system of reactions. These processes are outlined individually elsewhere in the book. Energy is actually recovered from several individual steps not shown in the figure and is not limited to the bottom stage indicated.

Anabolism may be regarded as the reversal of the general processes of the figure. Although not all metabolic reactions are individually reversible, the majority are, and in many of the other cases separate routes are known for going in both directions. Thus a diet deficient in certain amino acids may still allow the animal to draw on fat or carbohydrate and a nitrogen source for synthesis of the missing amino acid. Conversely, animals on diets of protein, vitamins, and minerals use part of the protein for energy, part as a source of necessary amino acids, and part for conversion to fats and carbohydrates.

The anabolism and catabolism of lipides, proteins, and nucleic acids are discussed in the next two chapters. General aspects of metabolism, respiration, and the energy content of foods are covered in Chapter 21.

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Lipide metabolism

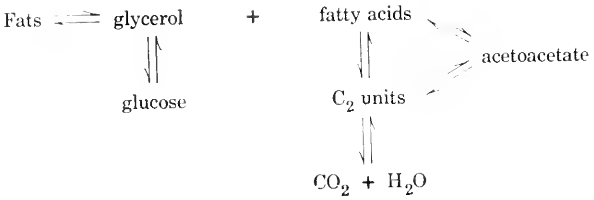
19

An appreciable portion of the body weight of a normal animal can be attributed to the presence of lipides. For example, 10 per cent or more of the body weight of a mammal is due to lipides, the majority of which is in the form of fats. As was stated in Chapter 13, body lipides are distributed in varying amounts in all organs and are stored in highly specialized connective tissue called depots. In these depots, a large part of the cytoplasm of the cells is replaced by droplets of lipide.

Body lipides serve as an important source of potential chemical energy. Each gram of fat, for example, yields more than twice as many calories as an equivalent weight of carbohydrate or protein. Moreover, the normal animal contains a greater quantity of easily mobilizable lipide than of carbohydrate or protein. In times of nutritional insufficiency, an animal can meet the endergonic requirements necessary for the maintenance of life by drawing on its lipide depots for reserve energy. Other functions of depot lipides are listed in Chapter 13.

FAT CATABOLISM

In anabolism, the liver serves as a temporary storage organ for fat while phosphorylation (phospholipide formation) is taking place. The phospholipides serve as the principal form in which fat is transported to the tissues. The liver also plays an important role in fat catabolism when carbohydrate metabolism is subnormal. The catabolic reactions taking place in the liver involve the hydrolysis of fats to glycerol and fatty acids. Glycerol is converted to dihydroxyacetonephosphate (see page 168) via formation of α-glycerophosphate and thus enters the carbohydrate metabolic scheme. Fatty acids, on the other hand, are degraded to several two-carbon units which either interact with oxaloacetate and are converted to carbon dioxide (page 171) or react with other two-carbon units to form acetoacetate which gives rise to substances known as ketone bodies. These reactions are summarized below.



The degradation of fatty acids proceeds stepwise by a series of reactions which removes two carbons at a time from the carboxyl end of the carbon chain. This catabolic scheme is known as β-oxidation.

β-Oxidation

In 1905, Knoop proposed a mechanism to explain the oxidation of fatty acids *in vivo*. He conducted a series of experiments in which ω-phenylaliphatic acids were fed to dogs, and the nature of the excretory products studied. He found that the aromatic nucleus resists oxidation in the body while the side chain is shortened by an even number of carbons. For example, β-phenylpropionic acid ⟶ benzoic acid, γ-phenylbutyric acid ⟶ phenylacetic acid, while phenylacetic acid itself is not degraded further. Both benzoic acid and phenylacetic acid are excreted in the urine in the form of their respective glycine conjugates, hippuric acid ($C_6H_5CO \cdot NH \cdot CH_2COOH$) and phenylaceturic acid ($C_6H_5CH_2CO \cdot NH \cdot CH_2COOH$).

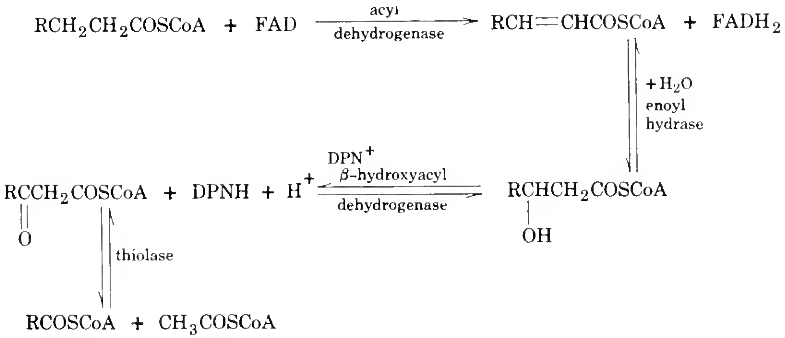
On the basis of Knoop's conclusions and a wealth of experimental evidence provided by many investigators since then, β-oxidation has become a firmly established metabolic pathway. Thus it is recognized that fatty acid oxidation occurs as the result of a series of reactions which effectively reduces the carbon chain two carbons at a time. The enzymes responsible for the various steps in the oxidation process have been isolated from liver mitochondria.

Recent investigations show that fatty acids must be activated before they are oxidized. Such activation involves the transformation of the free fatty acids to the corresponding acyl CoA derivatives. It is believed that the bulk of fatty acid activation takes place in various organs such as liver, heart, and kidney and is catalyzed by the thiokinases in the presence of ATP:

$$\mathrm{RCOOH + HS \cdot CoA + ATP \overset{\text{thiokinase}}{\rightleftarrows} RCO \cdot S \cdot CoA + AMP + HP_2O_7^{=}}$$

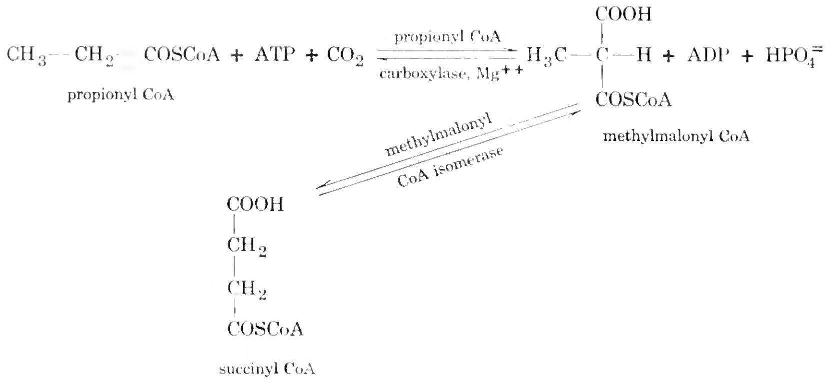
The acyl derivatives are joined to the CoA through the thiol group of the latter pyrophosphate.

After activation, fatty acids are oxidized in four steps involving reversible dehydrogenation, hydration, oxidation to ketone, and finally cleavage to yield the next shorter acyl CoA and acetyl CoA. A schematic summary of these reactions is shown below:



The metabolic importance and fate of acetyl CoA are discussed in Chapters 7, 9, and 20.

As shown above, the oxidation of a fatty acid containing an even number of carbons results in final degradation to acetyl CoA. On the other hand, the final oxidation residue of a fatty acid containing an odd number of carbons is propionyl CoA. The major pathway of propionyl CoA in the animal involves the conversion of this active fragment first to methylmalonyl CoA through the fixation of carbon dioxide and finally the isomerization of methylmalonyl CoA (isosuccinyl CoA) to succinyl CoA by an isomerase which does not seem to require any cofactors. These reactions may be summarized as follows:



$$CH_3{-}CH_2{-}COSCoA + ATP + CO_2 \xrightleftharpoons[\text{carboxylase, } Mg^{++}]{\text{propionyl CoA}} H_3C{-}\overset{\overset{\large COOH}{|}}{\underset{\underset{\large COSCoA}{|}}{C}}{-}H + ADP + HPO_4^{=}$$

$$\text{methylmalonyl CoA} \xrightleftharpoons{\text{methylmalonyl CoA isomerase}} \begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COSCoA \end{array}$$

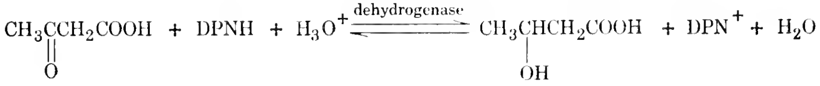
Besides being an intermediate in the Krebs cycle, succinyl CoA is used in a variety of metabolic reactions such as in the generation of an energy-rich pyrophosphate bond. Like acetyl CoA, succinyl CoA can act as an acylating agent. Thus, it is possible to succinylate amino groups in a fashion quite analogous to their acetylation by acetyl CoA. Complete information with regard to the importance of succinylation *in vivo* is lacking at the present time.

Ketone Bodies and Ketosis

The degradation of fatty acids in the animal body results in the production of some intermediates commonly called ketone bodies. These intermediate compounds, including acetoacetic acid, β-hydroxybutyric acid, and acetone, are all formed from acetoacetyl CoA through a variety of chemical reactions. For example, acetoacetic acid can be formed by a transacylation of acetoacetyl CoA with succinic acid.

$$\text{Acetoacetyl CoA} + \text{succinic acid} \xrightleftharpoons{\text{thiophorase}} \text{succinyl CoA} + \text{acetoacetic acid}$$

L-β-Hydroxybutyric acid is formed from acetoacetic acid in the presence of DPNH and β-hydroxybutyric dehydrogenase.



$$CH_3\underset{\underset{O}{\|}}{C}CH_2COOH + DPNH + H_3O^+ \xrightleftharpoons{\text{dehydrogenase}} CH_3\underset{\underset{OH}{|}}{C}HCH_2COOH + DPN^+ + H_2O$$

L-β-hydroxybutyric acid

Acetone formed *in vivo* from acetoacetic acid by decarboxylation may undergo further cleavage to yield a two-carbon acetyl and one-carbon formyl fragments. On the other hand acetone can be transformed to propanediol and thence to pyruvic acid, the metabolism of which has been discussed previously (Chapter 7).

Under certain conditions of abnormal metabolism, such as during starvation or diabetes, the concentration of ketone bodies increases to the point where the animal is said to have ketosis. When an animal is deprived of food for an extended period of time, the stores of glycogen in the liver are rapidly depleted and the animal is forced to depend largely on depot lipides for its energy. Accordingly the degradation of fatty acids in the liver takes place at a greatly accelerated rate with the result that there is formed an overabundance of acetoacetyl CoA, which in turn favors the formation of acetoacetic acid and its products, β-hydroxybutyric acid and acetone. Thus, ketosis develops.

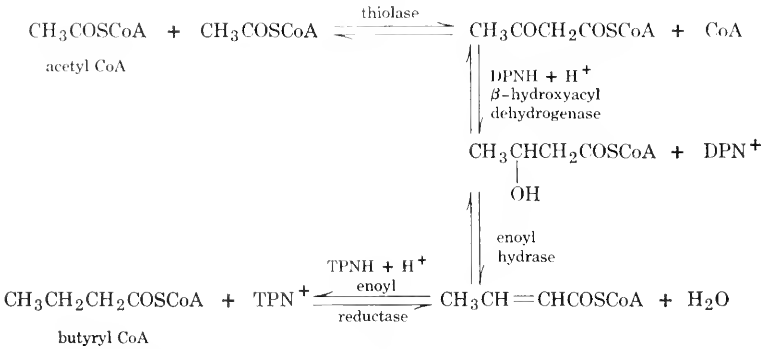
In diabetes, lipides are utilized for energy since normal carbohydrate metabolism is impaired owing to a lack of insulin. Hence once again fatty acid degradation takes place at a very rapid rate, producing ketone bodies in increasing amounts.

BIOSYNTHESIS OF FATTY ACIDS

Feeding experiments have shown that animals do not acquire fatty acids solely from dietary sources, but rather are capable of synthesizing some of them in the body. In fact it has been established that mammals can synthesize the major portion of the fatty acids required for growth and maintenance. In this way, the common saturated and monoethenoid fatty acids are formed in the animal body from a common precursor, acetyl CoA. Therefore, any dietary constituent or body tissue capable of producing acetyl CoA is at least theoretically a potential source of carbon for fatty acid synthesis (lipogenesis). Acetyl CoA is readily available from the metabolic reactions of carbohydrates, proteins, or fats. Although some amino acids such as leucine and alanine yield acetate directly, others are known to be glycogenic and thereby yield acetate by the carbohydrate route.

Mechanism of Lipogenesis

At first it was assumed that all the major steps outlined for fatty acid degradation were reversible, thereby affording a complete mechanism for the synthesis of fatty acids. However, it was soon discovered that lipogenesis differs from degradation in that the former has a specific requirement for TPNH as coenzyme for one of the reactions. Essentially, fatty acid synthesis may be described by 4 separate reactions, the first 3 of which are reversible reactions included in the degradation scheme. These reactions can be illustrated by starting with 2 moles of acetyl CoA and forming from them the 4-carbon intermediate, butyryl CoA.



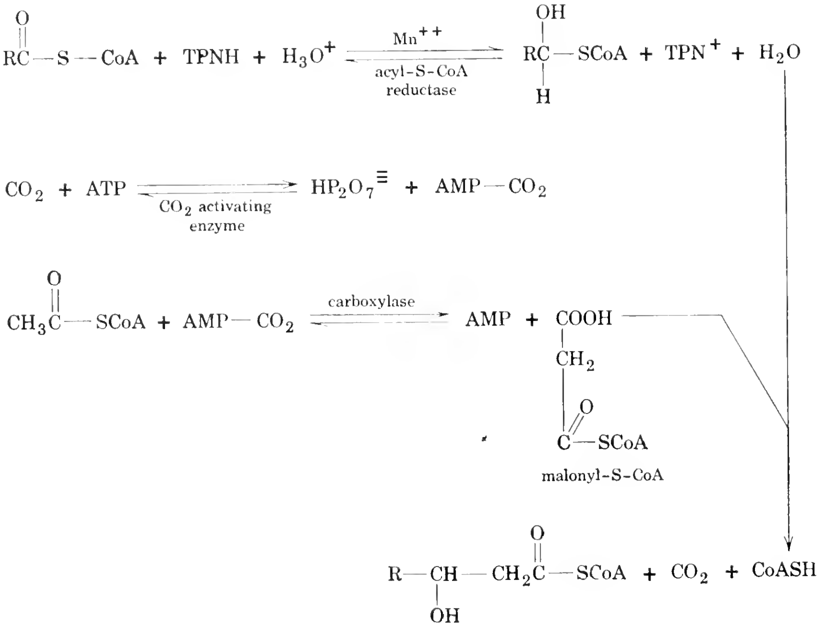
$$\underset{\text{acetyl CoA}}{CH_3COSCoA} + CH_3COSCoA \overset{\text{thiolase}}{\rightleftharpoons} CH_3COCH_2COSCoA + CoA$$

$$CH_3COCH_2COSCoA \xrightleftharpoons[\beta\text{-hydroxyacyl dehydrogenase}]{DPNH + H^+} CH_3\underset{\displaystyle OH}{\underset{|}{C}}HCH_2COSCoA + DPN^+$$

$$CH_3CHOHCH_2COSCoA \overset{\text{enoyl hydrase}}{\rightleftharpoons} CH_3CH{=}CHCOSCoA + H_2O$$

$$\underset{\text{butyryl CoA}}{CH_3CH_2CH_2COSCoA} + TPN^+ \xrightleftharpoons[\text{reductase}]{TPNH + H^+ \text{ enoyl}} CH_3CH{=}CHCOSCoA + H_2O$$

The above synthesis of fatty acids does not account for the ATP requirement in the reaction. Furthermore, in *in vitro* studies this synthesis does not operate efficiently beyond C-8 or C-10 fatty acids. Recently evidence for the existence of a modified aldol condensation has been presented by several laboratories. The suggested reactions can be illustrated as follows:



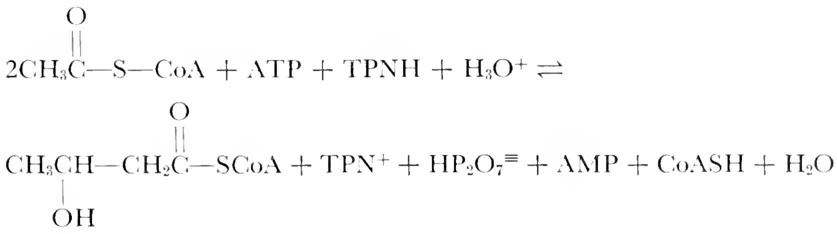
$$R\overset{\displaystyle O}{\overset{\|}{C}}{-}S{-}CoA + TPNH + H_3O^+ \xrightleftharpoons[\text{acyl-S-CoA reductase}]{Mn^{++}} R\underset{\displaystyle H}{\underset{|}{\overset{\displaystyle OH}{\overset{|}{C}}}}{-}SCoA + TPN^+ + H_2O$$

$$CO_2 + ATP \xrightarrow[\text{CO}_2\text{ activating enzyme}]{} HP_2O_7^{\equiv} + AMP{-}CO_2$$

$$CH_3\overset{\displaystyle O}{\overset{\|}{C}}{-}SCoA + AMP{-}CO_2 \xrightarrow{\text{carboxylase}} AMP + \underset{\text{malonyl-S-CoA}}{COOH{-}CH_2{-}\overset{\displaystyle O}{\overset{/\!/}{C}}{-}SCoA}$$

$$R{-}\underset{\displaystyle OH}{\underset{|}{CH}}{-}CH_2\overset{\displaystyle O}{\overset{\|}{C}}{-}SCoA + CO_2 + CoASH$$

The carbon dioxide addition serves to activate the methylene group, but carbon dioxide is lost again during the condensation reaction. The net reaction, assuming $R = CH_3$, is the same as the first mechanism proposed:

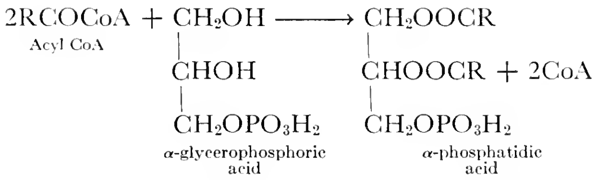


$$2CH_3\overset{\overset{\displaystyle O}{\|}}{C}\text{—}S\text{—}CoA + ATP + TPNH + H_3O^+ \rightleftharpoons$$

$$CH_3\underset{\underset{\displaystyle OH}{|}}{CH}\text{—}CH_2\overset{\overset{\displaystyle O}{\|}}{C}\text{—}SCoA + TPN^+ + HP_2O_7^{\equiv} + AMP + CoASH + H_2O$$

Clearly either pathway can repeat itself with the addition of more acetyl CoA to the butyryl CoA or reduction product. Thus, by repetitive steps, carbon chains can be increased 2 carbons at a time, producing longer-chain fatty acids at each turn. In order for the lipogenic process to operate, sources of DPNH and TPNH are needed. DPNH is produced by many dehydrogenations, but the majority of TPNH is formed during the dehydrogenation reactions of the isocitrate in the Krebs cycle and the phosphogluconic acid oxidative (pentose-phosphate) pathway (Chapter 9).

In the normal animal, the intermediate free fatty acids of varying chain lengths do not accumulate in significant amounts. Similarly, fatty acid chains are not increased 2 carbons at a time over an indefinite period of time. Rather it has been found that the reaction sequences described above are terminated by a reaction first described by Kornberg and Pricer. This reaction is specific for α-glycerophosphate and takes place preferentially with 16- and 18-carbon acid derivatives. On hydrolysis of the phosphatidic acid by phosphatase, a diglyceride is formed which will in turn react with another mole of acyl CoA to form a triglyceride.



$$\begin{array}{llll}
\underset{\text{Acyl CoA}}{2RCOCoA} + & CH_2OH & \longrightarrow & CH_2OOCR \\
 & | & & | \\
 & CHOH & & CHOOCR + 2CoA \\
 & | & & | \\
 & CH_2OPO_3H_2 & & CH_2OPO_3H_2 \\
 & \text{α-glycerophosphoric acid} & & \text{α-phosphatidic acid}
\end{array}$$

Fatty Acid Conversions

As pointed out in previous sections, an animal obtains fatty acids from the lipides included in the diet and from the biosynthesis of these compounds from acetyl CoA derived from carbohydrates and amino acids. Whereas the mixture of fatty acids obtained in the diet varies considerably with respect to chain length and unsaturation, the process of lipogenesis apparently favors the production of 16- and 18-carbon chains. Moreover it appears from evidence based on isotope data that saturated acids are formed in larger quantity and at a greater rate of speed than are unsaturated acids.

If isotopic stearic acid is fed to an animal a part of the isotope accumulates in oleic acid. In a similar fashion, feeding isotopic palmitic acid results in an accumulation of palmitoleic acid. Moreover, administration of isotopically marked palmitoleic or oleic acid results in the appearance of "tagged" palmitic and stearic acids in the depot fats. Thus it is evident that the animal is capable of introducing one double bond in the carbon chain between carbons 9 and 10 and also has the ability to convert such unsaturated acids to saturated ones. The liver is the major site for such conversions, although evidence is accumulating to indicate that these reactions can take place in other tissues as well.

Essential Fatty Acids

It is significant that, when isotopic stearic or oleic acid is fed to most animals, none of the isotope appears in linoleic or linolenic acid. This suggests that introduction of a second double bond does not take place, at least in some animals. Therefore, the inability to synthesize these polyunsaturated acids makes it necessary for such animals to ingest these compounds. Thus, linoleic, linolenic, and arachidonic acids are said to be essential fatty acids.

Although essential fatty acids cannot be produced *de novo* in any common species of mammal, there is evidence that certain transformations of these acids can be effected. For example, linoleic acid can be converted to γ-linolenic acid (6,9,12-octadecatrienoic acid), which by addition of 2 carbons and further dehydrogenation and isomerization yields arachidonic acid (5,8,11,13-docosatetraenoic acid). Although its role is not understood, pyridoxalphosphate appears to be essential in the formation of arachidonic acid from γ-linolenic acid.

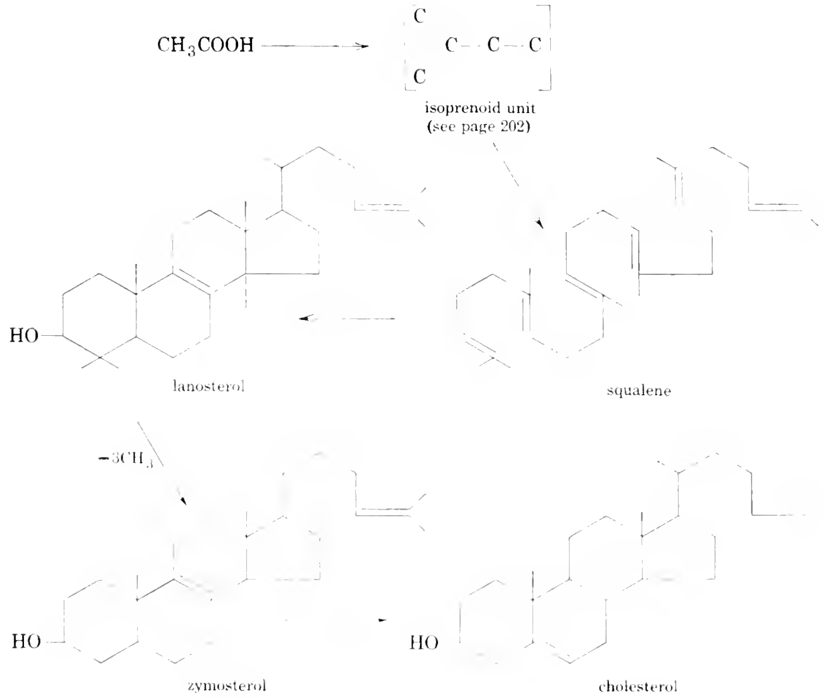
CHOLESTEROL METABOLISM

The sterol found in greatest abundance in animal tissues is cholesterol. Although foods such as eggs, milk, and animal fats furnish some cholesterol, the quantity ingested is not sufficient to account for the amount of this compound present in blood and body tissues. Therefore it is quite evident that cholesterol must be synthesized in the animal body.

Biosynthesis of Cholesterol

It has been established that all the carbons of cholesterol synthesized in the animal can be obtained from a common source, acetyl CoA. Although the liver is the site of the majority of cholesterol synthesis *in vivo,* it has been shown that this compound can be produced by a large variety of tissues. Moreover, many tissues, including liver, adrenal cortex, and arterial wall, are capable of stimulating the production of cholesterol *in vitro.* The use of such tissues *in vitro* shows that the cholesterol molecule can also be synthesized from acetoacetic acid, apparently without prior degradation to a C_2 unit.

Whatever the starting material, acetyl CoA, acetoacetic acid, or isovaleric acid, it is agreed that the formation of cholesterol proceeds through the condensation of acetyl groups to form an isoprenoid unit, which in turn serves as the skeletal material for the production of the hydrocarbon, squalene. Probably the first product of cyclization (ring formation) is lanosterol. At this point the hydroxyl group which ap-



pears in cholesterol is introduced. The enzyme system required for the cyclization and hydroxylation reactions is referred to as squalene oxidocyclase. These reactions also appear to depend on the presence of either TPNH or DPNH. The conversion of lanosterol to zymosterol requires TPNH and oxygen and has been demonstrated using rat liver homogenate. Both *in vivo* and *in vitro* studies show the possibility of conversion of zymosterol to cholesterol.

Cholesterol biosynthesis varies with the amount of cholesterol in the diet. Thus cholesterol biosynthesis is at a maximum when cholesterol-free diets are fed. On the other hand, cholesterol synthesis is sharply reduced during fasting and is drastically increased when the animal is fed a diet rich in carbohydrates.

Pathological Cholesterol Concentrations

About 80 per cent of the cholesterol metabolized by the body is converted into various bile acids (pages 91, 436). Under certain conditions, cholesterol crystals separate from the bile and produce the calculi commonly referred to as gallstones. The formation of calculi becomes clinically important if they descend into and block the biliary tract, preventing flow of bile.

Cholesterol-containing plaques sometimes form and attach themselves to the walls of the arteries, producing a characteristic lesion referred to as atheromatosis. At the present time, the mechanism and the cause of the deposition of such cholesterol plaques in man are quite controversial. Although evidence is increasing which suggests that inclusion of saturated fatty acids in the diet encourages formation of atheroma, there is no positive proof that substitution of the essential fatty acids for these saturated homologues will in fact alleviate or prevent atheroma formation. In some animals, pyridoxine deficiency results in the formation of atheromas. Since pyridoxine is required for the formation of the essential fatty acid, arachidonic acid, it is conceivable that faulty cholesterol metabolism can be related to a vitamin deficiency.

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Protein metabolism

20

Green plants and a variety of microorganisms utilize inorganic forms of nitrogen in synthesizing their proteins. The higher animals can also employ ammonium ion to some extent, but they all seem to need a certain proportion of organic nitrogen for protein synthesis. This requirement is at least partly satisfied by "essential amino acids" in the diets. Again, in this group of compounds, as in those of the earlier chapters, there is a major difference between the nutrition of animals and that of higher plants.

UTILIZATION OF PROTEINS

Animals regularly ingest proteins as components of the plant and animal materials eaten. In addition, the diet normally contains smaller quantities of free amino acids and peptides which supply a portion of the required organic nitrogen. Both the proteins and larger peptides must be hydrolyzed to amino acids and small peptides before absorption by the digestive tract. Only after these processes of digestion can the components of proteins be distributed and put to metabolic use by body tissues.

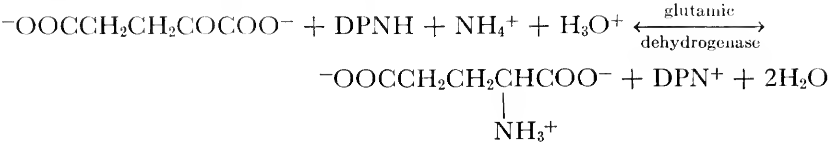
The products of the digestion of proteins are used in several ways. For example, certain amino acids may be converted into others needed by the animal but not obtained in sufficient supply from the diet. Or amino acids may be stripped of their nitrogen and oxidized to carbon dioxide and water for the energy available from such transformations. Still another fraction of the amino acids is condensed to form the proteins needed by the animal. These catabolic (breakdown) and anabolic (synthetic) processes are closely interlinked in a complex reaction network to make up the whole pattern of protein utilization. Some of the information concerning the systems involved is summarized in the following sections forming the remainder of this chapter.

Amino Acid Metabolism

Although a large number of amino acids are known to occur in cells, most of the biochemical effort has been expended on those occurring in proteins and on certain others having a close metabolic relationship. Even with this limitation the volume of information transcends the size of this book. Therefore, the presentation herein must be confined to particular schematic pathways and general features of processes rather than covering individual reactions. The pathways chosen are presented as partial examples of the reaction networks believed to exist. Such systems are still incompletely understood, but even the information available is too extensive for inclusion in a general textbook at the elementary level.

Perhaps the most extensively studied pattern of metabolic reactions of amino acids is that of Figure 20–1. This system of reactions accounts for synthesis of the L isomers of six amino acids commonly found in proteins and of others known only as metabolic intermediates. Moreover, most of the systems are either cyclic or reversible and provide mechanisms connecting with the tricarboxylic acid cycle, thus allowing use of these amino acids as sources of energy. Formation of urea by the "urea cycle" is believed to be the major source of this compound, which serves as the principal means employed by animals in excreting nitrogen.

Three of the reactions of Figure 20–1 are transaminations leading to the formation of alanine, aspartate, and glutamate by the mechanism of page 228. In addition, animals have the ability to utilize ammonium ion for the synthesis of glutamate according to the scheme shown. Although for simplicity the names have been omitted, the reactions of the figure are all enzymatic. In addition, it is probable that further study will reveal other connections and intermediates for processes written here as single reactions.



$$^{-}OOCCH_2CH_2COCOO^{-} + DPNH + NH_4^{+} + H_3O^{+} \xleftrightarrow[\text{dehydrogenase}]{\text{glutamic}} {}^{-}OOCCH_2CH_2\underset{\substack{|\\ NH_3^{+}}}{CH}COO^{-} + DPN^{+} + 2H_2O$$

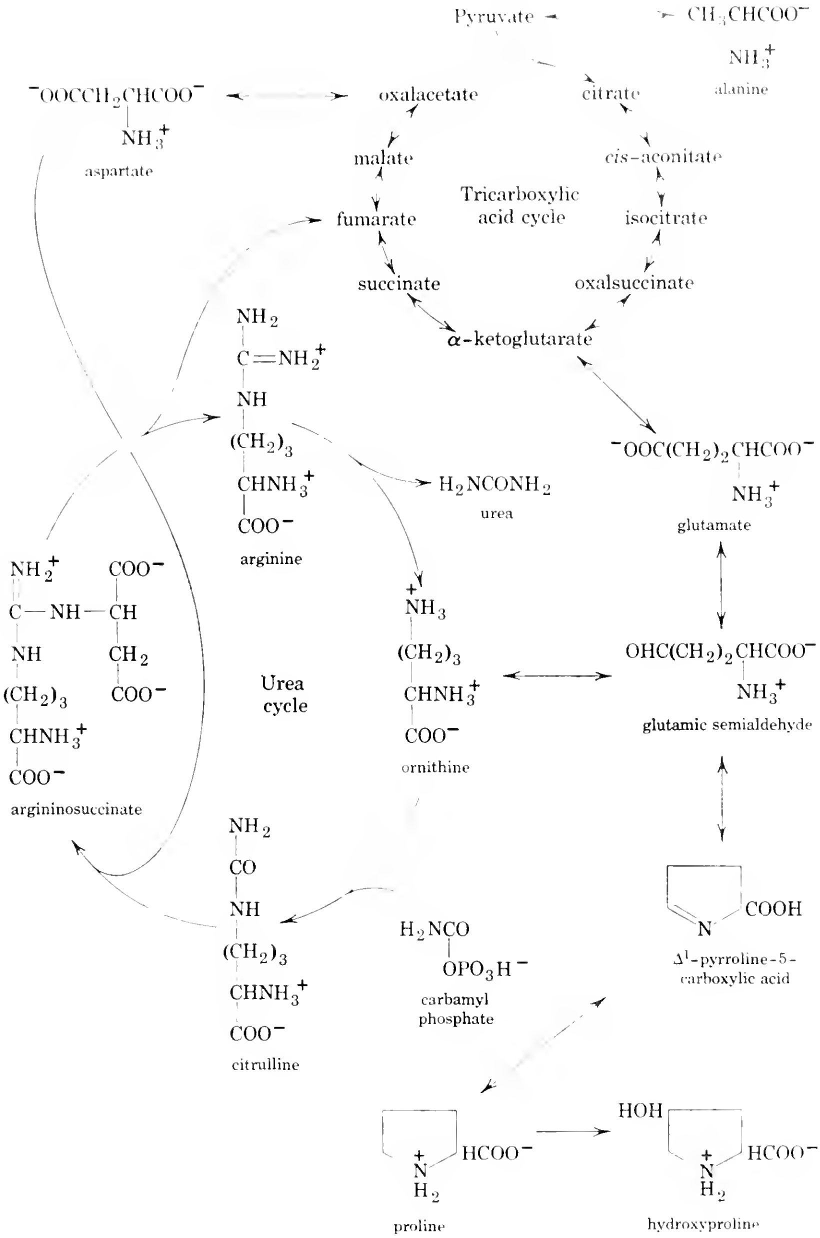
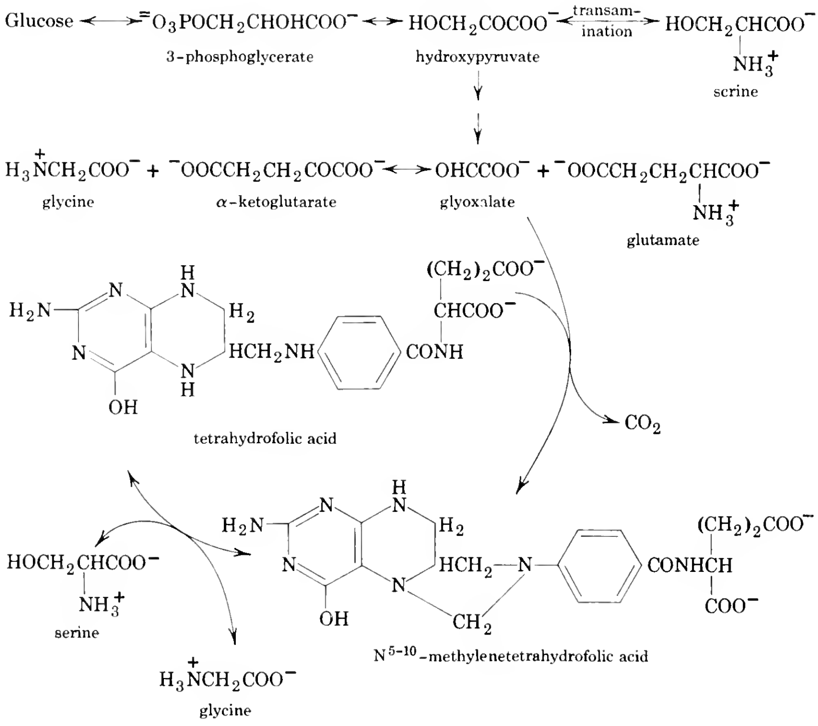


FIGURE 20-1. Metabolic transformations as of 1959 for several amino acids connected directly with the tricarboxylic acid cycle. Arrows with points on both ends signify demonstrated reversibility, while reactions represented by arrows with one pointed end are not known to be reversible.

Figure 20–2 summarizes the metabolic patterns of the three aromatic amino acids, phenylalanine, tyrosine, and tryptophan, commonly occurring in proteins. Since many steps are involved in the metabolism of these amino acids and since synthesis and degradation go by different routes, a number of known reactions have been omitted for simplicity. Both phenylalanine and tryptophan are nutritionally essential in certain mammals, perhaps in all. Therefore some of the synthetic steps leading to these two compounds may not occur in animals. However, it is not entirely clear whether mammals are devoid of all ability to make the so-called essential amino acids. Rather it is possible that a small synthetic supply exists but does not meet the demand. This point is considered again on page 478. The scheme outlined does occur in bacteria and might therefore be used indirectly by ruminants. The pathway shown probably accounts for the phenylalanine and tryptophan of plants.

The amino acids concerned in Figure 20–2 are deaminated and metabolized by routes connecting with the tricarboxylic cycle and leading ultimately to carbon dioxide and water. Certain important side products also appear. Of these, *p*-aminobenzoic acid has been



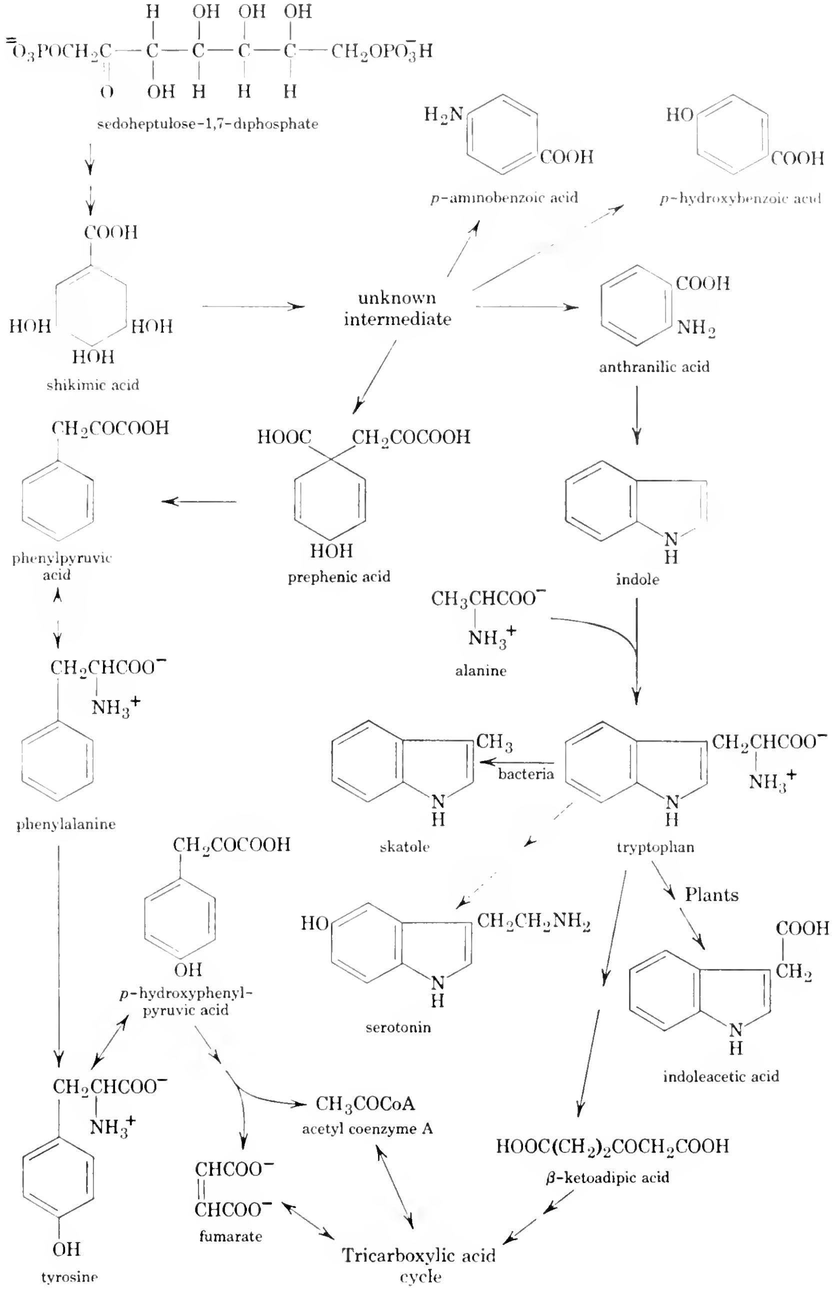


FIGURE 20-2. The metabolism of phenylalanine, tyrosine, and tryptophan summarized from information available in 1959. As customary in the current literature in this area, the acids are written and named in un-ionized forms. It is probable, of course, that all are ionized to a great extent inside cells. Pairs of arrows signify more than one known step with the reactions concerned omitted here for simplicity.

discussed in connection with folic acid. Intestinal bacteria convert tryptophan to skatole, a component of intestinal gases. Plants transform this same amino acid into indoleacetic acid, which serves as a growth regulator (page 281). Animals use tryptophan to make serotonin, which is a powerful vasoconstrictor and by its effect on blood vessels must help control blood pressure. Another pathway not indicated here leads from tryptophan to nicotinic acid and the important vitamin nicotinamide in those species synthesizing this vitamin.

The relationships between glycine, serine, threonine, and the sulfur amino acids are summarized in Figure 20–3. Glycine, together with other intermediates, is used by animal cells in making proteins, nucleic acids, purines and their nucleotides, porphyrins, and bile pigments. In addition, glycine and serine undergo transamination and degradation according to the mechanism shown on page 466.

This same reaction system also reveals the mechanism of the interconversion of glycine and serine, where the ($—CH_2OH$) of Figure 20–3 represents the tetrahydrofolic acid derivative shown above. The methyl groups required at other steps in the figure are shown as ($—CH_3$) and may come from this same transfer intermediate or methionine. The details of the transfer mechanism are still in doubt. However, where more than one methyl group is indicated the intermediate stages are known but omitted here to simplify the diagram. Ultimately all necessary methyl groups come from ingested methionine and serine or from glycine by way of glyoxalate.

Choline and ethanolamine are components of phospholipides and after being formed above become available for syntheses. Conversely the ingested supply can be metabolized when the synthetic needs are exceeded. The routes of metabolism yielding energy involve α-ketobutyrate from threonine and methionine and hydroxypyruvate or glyoxalate from glycine, serine, cystine, and cysteine. Serine and the other intermediates converted to it can be converted to either carbohydrates or fats for storage.

Other amino acids, including leucine, isoleucine, valine, lysine, and histidine, are abundant in the diets of animals and much is known of their metabolism. However, in the interest of space the reactions concerned have been omitted. It must not be construed that these amino acids are less important than the others which have been discussed. Rather the latter were chosen solely as examples to illustrate the complexity of amino acid metabolism and to reveal the close connection of all metabolites.

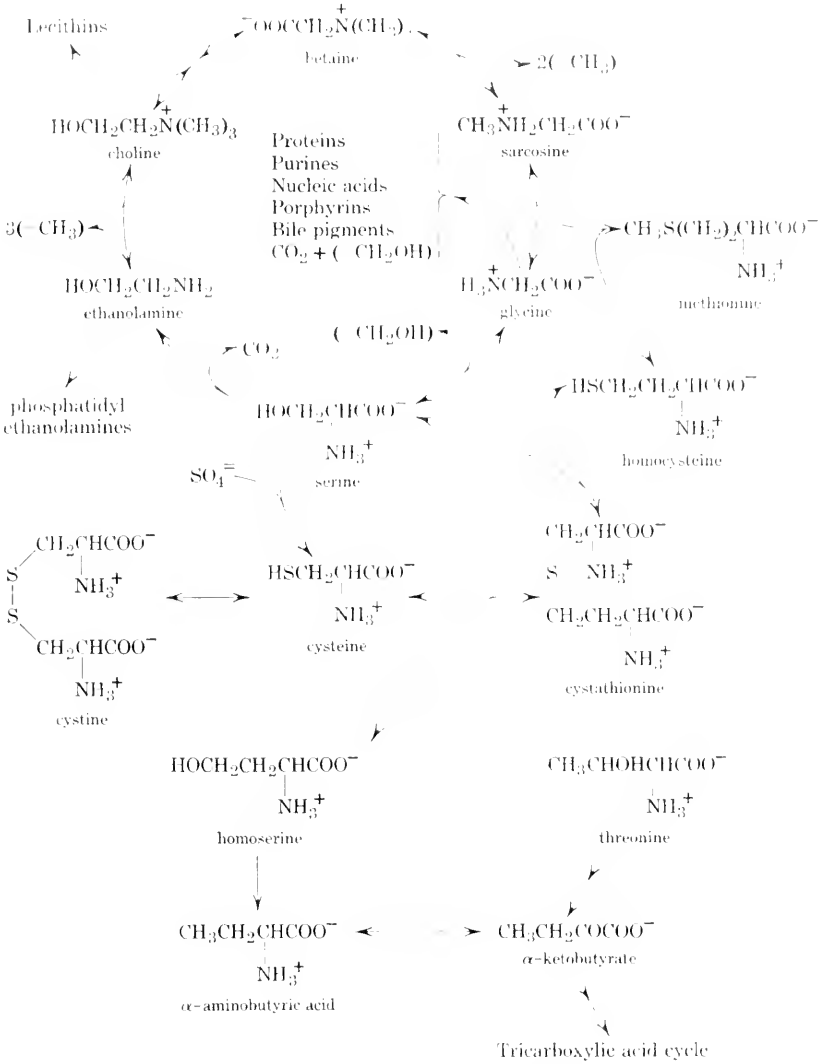
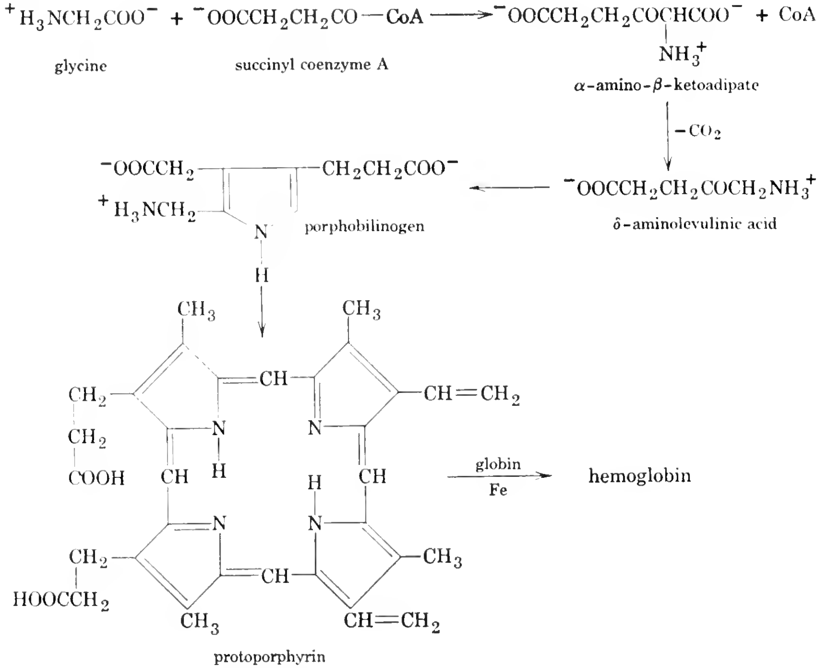


FIGURE 20-3. A 1959 summary of the utilization of glycine, serine, threonine, and the sulfur amino acids. The literature may be consulted for the intervening steps, enzymes, and cofactors omitted here.

Hemoglobin

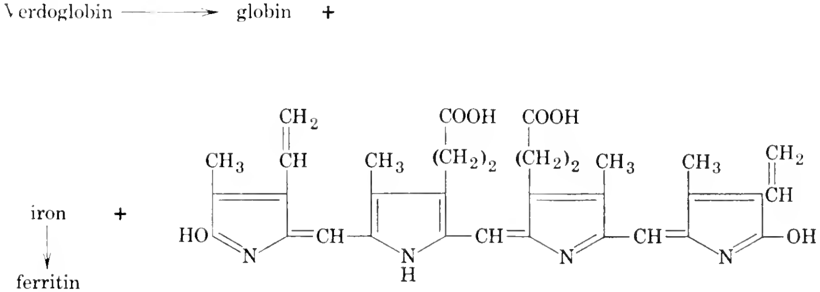
This protein is taken as an example of the complex proteins containing porphyrins that occur in the diet of both herbivorous and carnivorous animals. Furthermore, members of this group are important components of the animal itself both as the blood pigment hemoglobin and as respiratory proteins like catalase. Since red cells, for example, have a relatively limited life, some provision must be made for disposing of the hemoglobin liberated. Thus animals have both external and internal supplies of such materials to excrete or to make use of according to their needs and capabilities.

Most animals and birds appear to synthesize all the porphyrin they need for hemoglobin from glycine and succinyl coenzyme A. They do not utilize porphyrins from their diets to a significant extent. Isotope studies indicate that all the atoms of protoporphyrin come from glycine and succinate, revealing the very close connection between reactions already discussed and porphyrin synthesis.

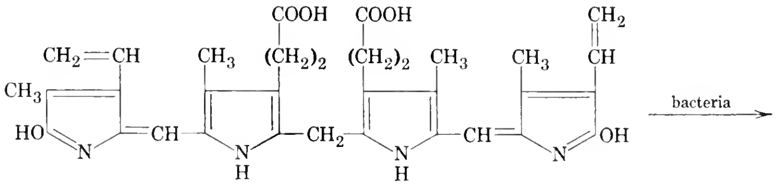


After an average life of about 125 days, erythrocytes are destroyed in human beings by cells in the spleen, liver, and bone marrow. The overall process leads to return of the globin to the amino acid pool and to excretion of degradation products of the porphyrin. Iron is carried by the plasma to the iron-storage system for later use. The porphyrin is converted to bile pigments which accompany the bile into the intestine and are excreted in the feces. Small quantities of bile pigments appear in the urine, amounting to 1 to 2 mg. daily for an adult human being compared to as much as 250 mg. in feces. Various abnormalities of liver function may block excretion of the bile and cause the accumulation of bile pigments, a disease called jaundice. Hemolytic jaundice, infectious hepatitis, cirrhosis of the liver, and obstruction of the bile passages are the most common ailments of this type.

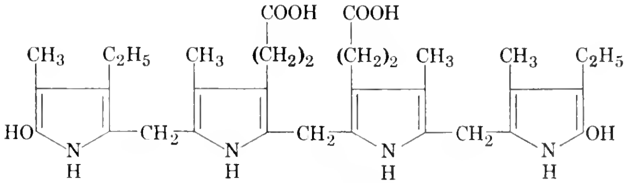
The bile pigments appear as follows. When the red cell is destroyed, the methene carbon (—CH group) is removed from between the right-hand pyrrole rings of the porphyrin structure. The resulting molecule is called verdoglobin and is still attached to the protein and still contains iron. This material then loses its iron and is split from the protein, forming biliverdin, a chain of pyrrole rings. This pigment is reduced to bilirubin (orange) which appears in the plasma in complexes with serum albumin and globulins. The liver traps the pigment and excretes it through the bile ducts into the duodenum of



biliverdin (green)

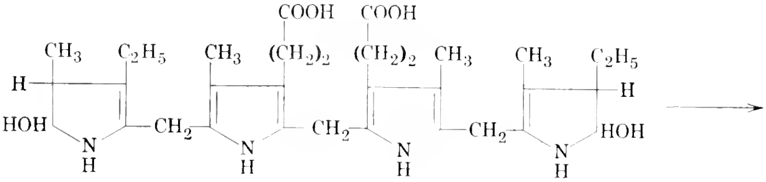


bilirubin

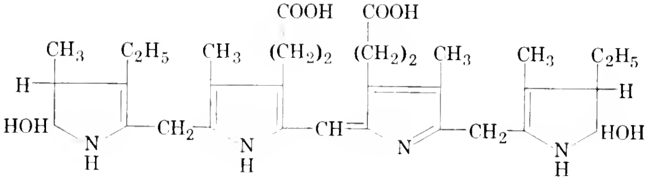


mesobilirubinogen

the small intestine. Bacteria of the intestinal tract then carry out a further reduction to mesobilirubinogen. Some of the latter material is excreted as such; most is probably reduced further to urobilinogen, which is partly reabsorbed, transferred to the liver by the plasma, and again eliminated in the bile. A part of the urobilinogen (also called stercobilinogen) is oxidized in the intestinal tract to urobilin (stercobilin) and is responsible for the brown color of feces. Small amounts of the urobilinogen recycled through the liver are excreted via the urine, where oxidation in light and air also leads to urobilin. Thus hemoglobin is degraded and eliminated from the animal body.



urobilinogen



urobilin

Perhaps other porphyrin-proteins are handled in a similar way, but it is not yet possible to trace them.

Nucleoproteins and Nucleic Acids

Animals make use of the components of nucleic acids and nucleoproteins in their diets. They also synthesize a supply of their own, so that these important materials need not occur in the food supply. On the other hand, the occurrence of nucleoproteins and perhaps nucleic acids in all living materials renders it advantageous to use them in metabolism.

Ingested nucleoproteins are split by the gastric fluids into nucleic acids and proteins. The latter are then digested as usual and the amino acids utilized as such or metabolized as outlined above. The nucleic acids pass into the small intestine, where they are probably hydrolyzed by pancreatic and intestinal nucleases into nucleotides. Nucleotidases and phosphatases then yield nucleosides and inorganic phosphate. After this process the nucleosides seem to be absorbed and transported to the liver, spleen, bone marrow, and kidneys, where a portion may be further split by nucleosidases to purines, pyrimidines, and ribose or deoxyribose. The pentoses thus become available to the systems of carbohydrate metabolism. The nucleases, nucleotidases, phosphatases, and nucleosidases concerned are poorly characterized but are probably mixtures of enzymes, each individually specific but together capable of hydrolyzing the naturally occurring materials.

The purines and pyrimidines not used for synthetic purposes un-

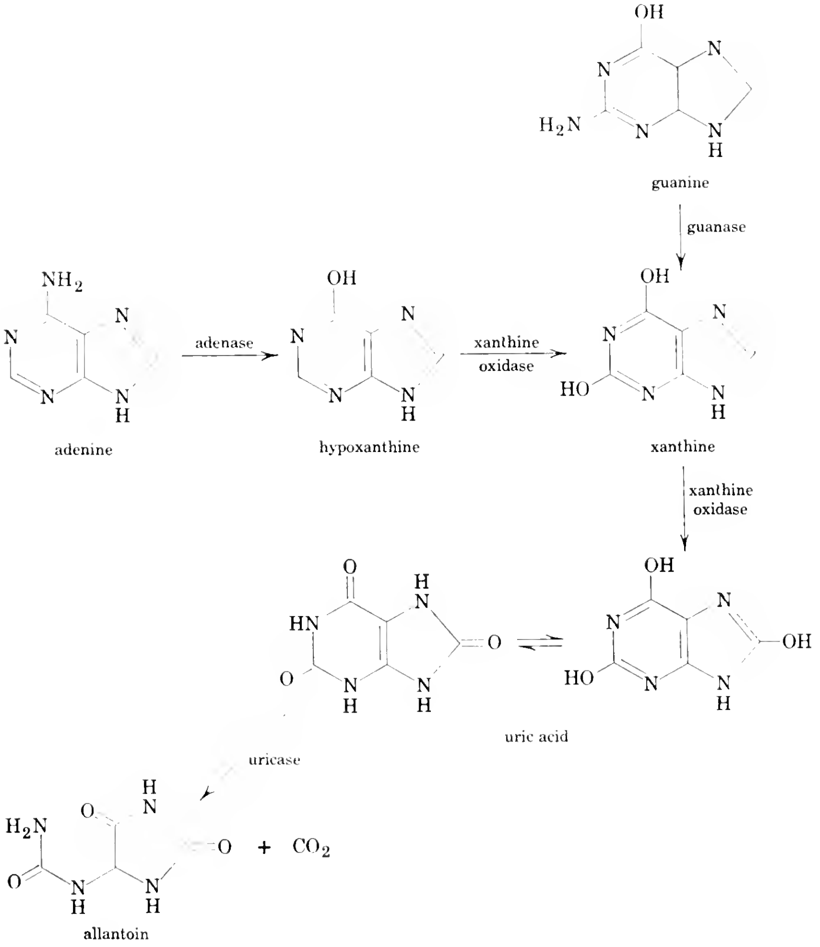


Figure 20–4. The catabolism of adenine and guanine.

dergo further transformations and are excreted, primarily in urine. Adenine and guanine, the purines of nucleic acids, are metabolized as in Figure 20–4. Primates (including man), birds, and some reptiles take the process only to uric acid and excrete that. Other mammals and reptiles excrete allantoin, and the remaining animals break purines down still further to urea in some cases and to ammonia in others.

By contrast, the free pyrimidines are degraded more extensively in mammals than are the purines. Although the reactions are largely unknown, the nitrogen appears as urea and ammonia. The carbon atoms are presumably utilized, but these reactions also are unknown.

Animals synthesize purine and pyrimidine derivatives from amino acids and various metabolic intermediates. These reactions have been extensively studied and elaborate pathways postulated. Rather than summarize these here, however, the reader is referred to recent reviews on the subject. Once the nucleotides are formed, they are converted to nucleic acids. The necessary condensation reactions have been carried out, using extracts of cells and high-energy compounds to drive the reaction. Although the process is complex, its isolation from the living cell assures rapid progress in understanding many of the individual steps. Great advances from work on the synthesis of purines, pyrimidines, and all intermediates leading to nucleic acids can be expected in the next decade.

Protein Nutrition

The class of foodstuffs called proteins has long been recognized as an essential dietary component for animals. As commonly the case for other nutrients, the need for protein varies with the species and to some extent the individual. In part, the requirement for protein is a requirement for nitrogen, since dietary protein contributes the major portion of the ingested nitrogen available to the animal.

Early workers knew that digestion hydrolyzed much of the protein to amino acids. Yet they apparently felt that the protein also played a role distinct from that of the amino acids themselves, since animals failed to grow normally on diets containing amino acids in place of proteins. Later discovery of threonine in proteins and its incorporation into experimental diets avoided part of the difficulty encountered previously. Subsequent work on vitamin B_{12}, which often accompanies animal proteins, further clarified the problem and supported the concept dating to about 1900 that proteins served solely as sources of certain important amino acids. More evidence has been accumulated from the numerous studies showing that human beings, other mammals, and birds can grow or maintain themselves when fed amino acids in place of proteins if the other known necessary factors are supplied.

On the other hand, some of those microorganisms requiring external supplies of amino acids grow best when certain peptides are present. This rather widespread observation indicates that particular peptide linkages are important to cells having limited capacities to make them. Therefore, inclusion of particular peptides in the medium permits more rapid growth. Although the best evidence of this type exists only for microorganisms, there are similar indications for animals in reports that growth is better on certain proteins than it is on mixtures of amino acids matching the proteins in composition. Such work can be interpreted in several ways.

First, particular peptides may stimulate growth when fed to supplement the synthetic capacity of the animal. Second, the protein may be contaminated with some known or unknown factor needed by the animal and not otherwise supplied adequately in the diet. Mixtures of isolated amino acids would be deficient in such materials. Third, the analysis of the amino acid content of the protein may be inaccurate or incomplete, making the mixture an imperfect match. Most analyses require hydrolysis of the protein, and several of the amino acids are partly destroyed. Evaluation of this effect is difficult and may lead to underestimation of amounts present. Furthermore, there is reason to suspect that ornithine and citrulline may occur in at least some proteins, but direct evidence is lacking. Hence there may actually be components of whose existence we are unaware. As a consequence, it cannot be assumed that proteins serve solely as dietary supplies of amino acids.

In blood transfusions advantage is taken of the fact that intact proteins can be used directly when injected into the circulatory system. Thus the protein taken from another individual can replace that of a person or animal that accidentally loses blood. The same principle is involved in the use of insulin by diabetics. However, there are limitations to the practical application of this idea because injection of proteins from other species or even other individuals can cause serious physiological reactions. Awareness of this possibility has led to the typing of blood for transfusion to assure use of blood that will not harm the recipient.

The problem of food allergies probably is related to this interference by foreign proteins. Proteins of certain kinds apparently pass directly or as large fragments through the intestinal wall into the blood stream. These foreign macromolecules then produce the undesirable responses termed allergies in individuals absorbing such protein materials from particular foods. Since food allergies apparently follow the absorption of proteins, it is possible that the absorption of peptides is quite common and that some of these are used directly in protein syntheses, reducing the number of steps required.

Protein Allowances

Since proteins serve as the major sources of nitrogen, they must be ingested regularly. This continuing need is perhaps more acute than for either fats or carbohydrates, for as outlined on page 480 animals are incapable of large-scale storage of proteins. Moreover, fats and carbohydrates obviously cannot be converted to amino acids and proteins without a supply of nitrogen. Therefore, it is probably more important that proteins be eaten regularly than carbohydrates or fats since the last two are more readily stored and interconverted. A consumption of protein beyond the minimum requirement does not seem at all harmful for the extra amino acids are metabolized by the several routes to ammonium ion, urea, carbon dioxide, water, and useful energy. As expected, the needs depend on the species, individual size, and physiological activity.

Daily allowances for adult human beings have been set at 1 g. of protein per kg. of body weight. This level amounts to about 70 g./day for men near the average weight of 155 lb. and provides an excess of perhaps 50 per cent above the minimum requirement. Some excess is important to allow for differences in physical activity and especially for differences in the effectiveness of different proteins. The ration of about 60 g. of protein daily (dry-weight basis) for women should be increased to 85 and 100 g. daily during pregnancy and lactation to meet the elevated rates of protein synthesis.

Children have relatively higher requirements because of their bodily growth. Although not based on extensive experiments, the recommended levels have been set at 3.5 g./kg. of body weight for children up to 1 year old. Then the food should contain 40 g. daily to 3 years of age with increments of 10 g./day added for each 3-year increase in age, bringing the level to 70 g. daily at the age of 12. Girls of 12 to 20 and active boys of the same age have allowances set, respectively, at 75 to 80 and 85 to 100 g. of protein daily.

Recommended protein levels for cattle range from 0.30 lb./day for 50-lb. calves to 1.2 lb. for pregnant dairy cows and 1.4 lb. daily for rapidly gaining beef cattle with weights of 800 lb. or more. The detailed allowances are listed in the Appendix.

Allowances for swine are exceptionally high because this species has an unusually rapid rate of growth and range from 0.6 to about 2 lb. daily. The Appendix summarizes the details for man, cattle, horses, sheep, swine, chickens, and turkeys. In certain cases the allowances are expressed as percentages of the ration, which in part compensates for differences in body weight since larger individuals normally eat more.

Starvation is the state arising from a lack or partial deficiency of food. Some of the physiological changes are due at least in part to a shortage of protein. Thus protein deficiencies are associated with famines. In some areas of the world a more specialized situation is common among people in the lowest economic groups. Although the total food intake is usually low, the protein levels are relatively lower still, producing a disease called kwashiorkor with poor growth, anemia, lack of pigmentation, low blood-protein levels, diarrhea, reduced pancreatic function and intestinal digestion, fatty livers, and wasting ending in death in 30 to 90 per cent of untreated children. Although not limited to children, the disease is most common among them because the additional needs for growth raise their relative protein requirements and because after breast feeding they may be given only cereals such as taro, corn, and millet.

Kwashiorkor is perhaps the most widespread nutritional disease (other than starvation itself) now taking human lives. It is common in tropical America, central and southern Africa, and India. Unfortunately, severe cases do not respond to the feeding of protein. Body proteins are at such reduced levels that dietary proteins are not digested and thus cannot be used effectively. However, the administration of amino acids and protein hydrolysates is quite effective and after a time these products may be replaced with ordinary proteins. Since foods high in protein are normally the most expensive, the problem is an economic one to a great extent.

Nitrogen Balance

As indicated earlier, erythrocytes, other cells, and many body proteins are continually being broken down. Part of the nitrogen present is excreted as urea, bile pigments, and uric acid (or allantoin), and as bacterial nitrogen in the feces. This loss cannot be continued without replacement if the animal is to remain healthy. Hence a normal individual must consume nitrogen equivalent to that excreted. Daily fluctuations are probably not important, providing income and outgo are equal over longer periods. When they are, the animal is said to be in nitrogen balance, meaning that he eats the same amount of nitrogen as the combined losses in urine and feces.

Normal adults on normal diets thus should always be in nitrogen balance. If physical activity increases the demand for nitrogen needed in repairing muscle tissue, appetite increases too. When an individual increases his food intake without a change in actual need, the excess protein is stored to a limited extent at first. Thereafter, the excess is converted to fat for storage and the nitrogen eliminated. Conversely, a limited decrease in protein consumption first eliminates the conversion to fat and reduces the limited storage, so that intake and output again equate but at a somewhat lower level.

However, when protein intake is sharply curtailed, nitrogen excretion cannot decrease correspondingly and continues to exceed the supply. Such a state, called negative nitrogen balance, arises during starvation, dieting to reduce total body weight, wasting illnesses like fevers, and improper diets deficient in materials essential for protein utilization. Obviously a prolonged negative nitrogen balance will be harmful and may lead to kwashiorkor, for example.

On the other hand, young growing animals ingest more nitrogen than they excrete, using the difference for cell multiplications and tissue increases. This situation is called positive nitrogen balance. Normal adults do not show this behavior. However, positive nitrogen balance does occur during pregnancy, recovery from loss of blood or wasting diseases, wound healing, or very briefly when the level of protein intake rises appreciably. After parturition or the repair of tissue is completed, the individual returns to nitrogen balance until the next departure from normality causes a shift in either the positive or the negative direction.

Essential Amino Acids

For experimental purposes animals have often been fed diets containing all known dietary factors but with only a single protein. When the level of the protein in such a diet is varied, that level just sufficient to allow nitrogen balance is taken as the quantity required for well-being. When different proteins are studied in this way, different minimum amounts are required. This variation is now attributed to differences in protein structure, and most of it to variations in the amino acid composition of the proteins.

Studies on the nature of this effect employ mixtures of the amino acids found in proteins added to the otherwise complete rations instead of protein. By omitting the amino acids one at a time, it is possible to discover those that must be supplied as distinguished from those that can be synthesized by the test animal. Furthermore, variations in the levels fed will also fix the minimum requirement for each essential amino acid.

Naturally there are complications. To some extent the results depend upon the physiological state of the animal. Requirements for the growth of young animals differ from those for the maintenance of adults. Also requirements for pregnancy, lactation, recovery from illness, and wound repair will differ from those for normal animals. In fact, there is evidence that certain amino acids may not be essential in the diet in some cases but may be required in others when the synthetic capacity is not sufficient to meet special demands. In addition, there are species differences even among mammals. Finally there are purely experimental problems. A diet containing only the essential amino acids in the amounts minimally required is inadequate for any purpose. Such a diet does not contain enough nitrogen to permit a suitable synthesis of those amino acids the animal can make. Therefore minimum levels of essential amino acids must be met for physiological needs, and minimum total nitrogen (or protein) levels must also be met by the diet.

Table 20–1 lists the amino acids essential for the growth of infants and rats. Chicks require the same compounds, with the addition of glycine according to some reports. A requirement for glycine is surprising in view of the presumably widespread metabolism of

TABLE 20–1. Essential Amino Acids and the Levels Required Daily

	Human Beings, mg./kg. of body weight		Rats, % of ration for Growth of Young
Amino Acid	For Growth of Infants	For Maintenance of Nitrogen Balance	
Arginine	130	0	0.2
Histidine	60	0	0.4
Isoleucine	20	20	0.5
Leucine	430	30	0.8
Lysine	170	20	1.0
Methionine	90	30	0.6
Phenylalanine	170	30	0.7
Threonine	90	10	0.5
Tryptophan	30	10	0.2
Valine	160	20	0.7

glycine by the mechanisms of Figure 20–3. Neither dogs nor mice require arginine, at least under some conditions. The influence of physiological state is illustrated in Table 20–1 by comparison of the values for human beings. Arginine and histidine are not essential in the diets of normal adults. When a state of positive nitrogen balance is needed temporarily, a dietary requirement for these two amino acids might be expected. The values of the table cannot be taken as absolute since there appear to be rather large differences between apparently similar and normal individuals. Thus allowances have been suggested that are greater by a factor of two than the minimum values tabulated here.

Since proteins differ considerably in the relative proportions of the amino acids they contain, they differ also in the quantities needed to supply the required levels of all the essential amino acids. Table 20–2 compares proteins from several sources with regard to maintenance of nitrogen balance in normal men. These requirements are subject to all the conditions discussed above as affecting the needs for essential amino acids. Comparisons of the levels in the table reveal that it is advantageous to supplement vegetable diets with animal proteins. Good feeding practice in the livestock industries recognizes this fact and depends upon waste animal materials from meat-processing and food-handling operations as cheap sources of animal proteins.

TABLE 20–2. Requirements of Various Proteins for Nitrogen Balance in Normal Men

Protein Source	Requirement, g./kg./day	Protein Source	Requirement, g./kg./day
Beefsteak	0.27	$\frac{2}{3}$ Mixed vegetable +	
Whole egg	0.28	$\frac{1}{3}$ mixed meat	0.39
Corn germ	0.30	General American diet	0.39
Haddock	0.31	Potato	0.42
Cottonseed flour	0.33	Soybean + white flour	0.43
Yeast	0.34	All-vegetable diet	0.46
Milk	0.35	Wheat flour	0.55
Soybean flour	0.36	White flour	0.60
Beef	0.38	Whole wheat bread	1.0

Protein Storage

When protein intake is increased, excretion lags somewhat behind ingestion, indicating that nitrogen is being stored. After a time the steady state is re-established with equality between intake and output. In the meantime, however, the liver, kidneys, and intestinal tissues have increased in both weight and protein content, and the plasma proteins have also increased in quantity. Conversely there is a reduction in the protein of these materials to a lower level when protein intake is reduced.

Starvation amplifies this effect, and the voluntary muscles begin to waste away. Heart muscle, on the other hand, is more resistant to decrease and loses protein more slowly. At the beginning of fasting, the glycogen reserves are used and little nitrogen is excreted. As a result, the carbohydrate is said to have a sparing action for protein. Limited ingestion of fats has a similar effect and delays the marked losses of body protein. However, the ingestion of large quantities of fat with little or no nitrogen actually accelerates the loss of nitrogen from the body. Thus the storage or retention of protein depends upon the levels of both protein ingestion and of carbohydrates and fat in the diet. Although there are important metabolic interactions, fat and carbohydrate cannot replace protein, and a balanced ration is necessary.

EXCRETION OF NITROGEN

Excretion of various nitrogenous compounds has already been referred to, including urea, uric acid, allantoin, ammonium ion, and bile pigments. These compounds along with others appear in either the urine or feces to account for the excretion of nitrogen. Minor additional losses probably occur with perspiration and shedding of cells from the skin surface. These latter factors will not be considered further.

Urine

Mammals excrete most of the nitrogen as soluble compounds in the urine. The most abundant of these are listed in Table 20–3. Urea is the major end product of nitrogen metabolism in all mammals, although much less important in other animals. Its concentration in urine is dependent upon the level of nitrogen intake and accounts for the excretion of excess nitrogen after unnecessarily great ingestion of protein. If protein intake is below the required level, urea excretion is markedly reduced.

Urea is formed by the urea cycle (page 465) and dialyzes into the blood. The kidneys remove it from the circulating blood and con-

TABLE 20–3. The Principal Nitrogen Compounds in Urine of an Adult Human Being

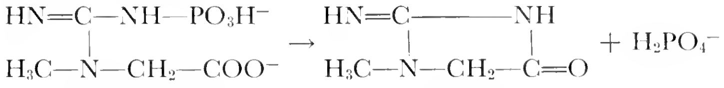
Component	Quantity, g. of N per day
Ammonium ion	0.4 –1.0
Amino acids	0.08–0.15
Creatinine	0.3 –0.8
Hippuric acid	0.04–0.08
Peptides	0.3 – 0.7
Urea	6 –18
Uric acid	0.25– 0.7

centrate it in the urine by a large factor, equal in human beings to about 60 times the concentration in the plasma. Various abnormalities render the kidneys incapable of this power of concentrating urea, which then accumulates in the blood. Although urea is not toxic, abnormally high concentrations of it indicate kidney malfunction and are usually accompanied by toxic symptoms due to other urine components. Such diseases are known as uremias.

As with other components, the quantity of ammonium ion excreted daily varies by a factor of 2 to 3, depending on the diet and the individual. Variations outside this range indicate a disturbance of normal function. Originally it was supposed that the ammonium ion appeared after decomposition of urea. However, tracer studies with isotopes show that this is not the case but that the ammonium ion results from deaminations of amino acids. Since ammonium ion is used in the formation of urea, it must be assumed that the urea cycle does not utilize all the NH_4^+, or that the ion appears in tissues not synthesizing urea, or perhaps both.

Uric acid in man and birds and allantoin in mammals other than primates appear after the breakdown of purines. Hence the levels depend upon the intakes of purines and nucleic acids. However, the levels ordinarily do not drop below the normal range even on a purine-free diet. Since animals also make their own purines from amino acids, synthesis and degradation seem to occur at a constant rate as long as ingested amino acids are available. Aside from dietary effects, uric acid may increase in the urine of leukemia patients. There may be enormous excretions when certain drugs are used to treat arthritis. Since uric acid and its salts are not very soluble, these compounds can crystallize in the urinary tract. On the other hand, a reduced excretion of uric acid is believed to precede an attack of gout, which is attributed to an accumulation of sodium urate in the affected joints.

Creatine and ornithine are both formed from arginine and glycine. Creatine phosphate is important in muscle and appears in the urine in small amounts. Creatinine is the normal urinary waste product probably derived from creatine phosphate by the spontaneous irreversible process shown here:

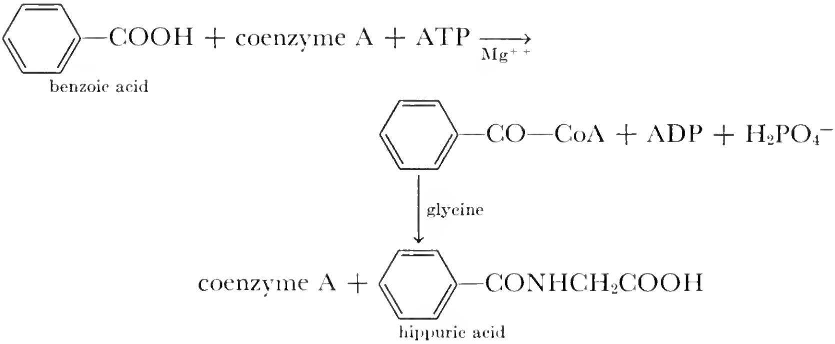


$$\begin{array}{l} HN{=}C{-}NH{-}PO_3H^- \\ \quad\;\; | \\ H_3C{-}N{-}CH_2{-}COO^- \end{array} \longrightarrow \begin{array}{l} HN{=}C{-\!-\!-\!-}NH \\ \quad\;\; | \qquad\quad\;\; | \\ H_3C{-}N{-}CH_2{-}C{=}O \end{array} + H_2PO_4^-$$

creatine phosphate creatinine

Although Table 20–3 reports a range of levels for the excretion of creatinine, the value for a given individual is remarkably constant. The actual level depends upon the quantity of muscle tissue possessed by the individual but upon no other known factor, including muscular activity. Hence each person or animal has a characteristic excretory level which fluctuates very little in the absence of any condition that causes muscle wastage. Starvation, diabetes, fever, hyperthyroidism, and progressive muscular dystrophy lower the excretion of creatinine. In such situations it is common to find creatine in amounts compensating for the decrease in creatinine.

Hippuric acid appears in urine to the extent of about 0.7 g. daily. It is formed during the detoxication of benzoic acid, which occurs in fruits and berries and is widely used in foodstuffs as a preservative. The reaction occurs in liver and is sufficiently characteristic to serve as a test of liver function.



Amino acids are also normal constituents of urine but are limited in quantity (Table 20–3) and not all are found. Since these substances occur in plasma, presumably they may be dialyzed from the blood and incompletely reabsorbed by the kidneys. Although little work has been reported on the point, observed levels should parallel changes in protein intake and metabolism. One hereditary disease called cystinuria leads to the urinary excretion of relatively large quantities of certain amino acids, including ornithine, cystine, arginine, lysine, and isoleucine. Since cystine in particular is not very soluble, stones in the urinary tract are common in persons having this disease. Other animals are subject to the same sort of disability.

A greater quantity of nitrogen appears in peptides than in free amino acids. However, little is known of the origin and amino acid compositions of these materials. Traces of proteins are also normal, mostly as serum albumin and globulins and material from the lining of the urogenital system. A variety of pathological states causes marked increases in the protein level, largely as serum proteins. This change is due either to modifications in permeability or to interference with reabsorption. At present a preferred explanation cannot be chosen.

Small amounts of other compounds also occur in normal urine. Some of these include the water-soluble vitamins, peptide hormones, organic sulfates, glycosides, and steroids. Many of the mineral materials discussed elsewhere also are excreted in the urine.

Feces

As formed in the normal intestinal tract, feces consist primarily of water, undigested fragments of food, indigestible materials, bile pigments, enzymes, leucocytes, bacteria, certain mineral materials, and mucus and cellular debris from the wall of the intestine. The mixture leaving the small intestine is rather fluid, a state favoring enzyme action and the intestinal absorption of nutrients. In the caecum and colon, much of the water is absorbed, reducing the total weight to a half or a third of the original value.

The indigestible materials are rather limited in amount since proteins, lipides, and most carbohydrates are efficiently hydrolyzed and the components absorbed. In ruminants and other herbivores like horses, much of even the cellulose is digested. However, lignin and some cellulose are excreted by these animals and by the omnivores man, rat, and pig. Carnivores normally ingest very little cellulose. Other materials, like hair, cartilage, soil, seeds, and unchewed pieces of comparatively hard and insoluble foodstuffs, make up a varying percentage of the bulk.

The other major component of feces consists of bacteria, which may represent a fourth of the total dry weight. A variety of organisms occur, some of them pathogenic. *Escherichia coli* is the most abundant bacterial species in man. Since ruminants depend upon extensive bacterial populations in their rumen digestive processes, many of these special organisms are subsequently excreted. It is believed that practically all the nitrogen of feces occurs in the bacteria and amounts to a total of approximately 0.7 to 1.0 g. daily. By comparison with Table 20–3, this quantity is seen to be a rather small fraction of that in urine.

Other nitrogeneous materials occur to some extent, but most of these are attacked by the intestinal bacteria and the nitrogen assimilated. Bile pigments and porphyrins are at least partial exceptions. Of the latter, hematin is probably the most abundant (especially in the carnivores) since ingested hemoglobin gives rise to this indigestible substance. The dark color of large quantities of hematin may make feces nearly black. Bleeding of the upper portions of the gastrointestinal tract produces this same coloration, also due to hematin. Peptic ulcers and cancers are the commonest causes. Bleeding in the lower part of the tract leads to bright red oxyhemoglobin excreted as such because it does not encounter the digestive processes.

PROTEIN SYNTHESIS

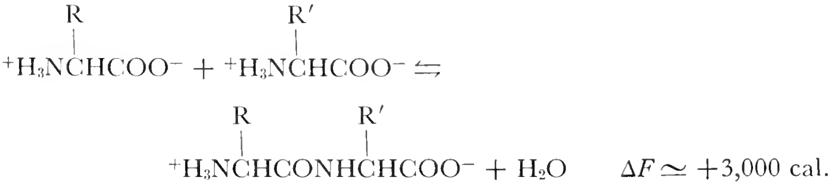
The mechanism of the biosynthesis of proteins is one of the great unsolved problems of biochemistry. Since living forms all synthesize proteins as one of their principal classes of components, the processes become important for quantitative as well as qualitative reasons. The importance of protein synthesis is further emphasized by enumerating some of the diverse roles played by these materials. In addition to acting as food for the newborn, proteins serve as catalytic, skeletal, genetic, hormonal, and transporting materials of diverse types. The total protein is a large fraction of the dry matter of any cell or any animal.

Because of the great fundamental interest in it and the probable value to practical medicine, the mechanism of protein formation has been widely sought. In spite of this activity, little has been learned beyond the general outlines and even these are still in doubt in many cases. Much of the difficulty seems to arise from two factors; first, the great molecular size and complexity of proteins, and, second, the specificity of their action, which is dependent in turn upon specificity in structure.

Requirements

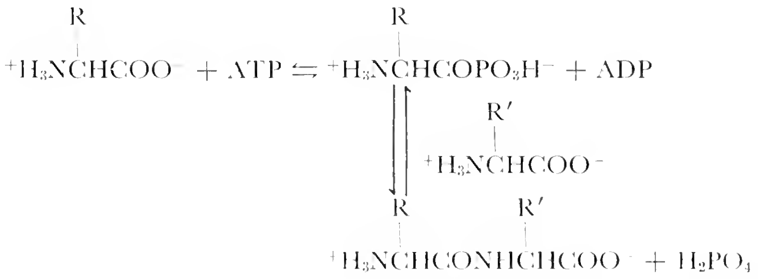
The overall problem of protein biosynthesis appears to resolve into three general requirements. The first of these involves the necessary raw materials, the amino acids. Since proteins are readily hydrolyzed to amino acids and obviously are combinations of these materials, at least to a large extent, it has always been assumed that amino acids are actually combined somehow in forming proteins. The other possibility, that another type of unit condenses and is later converted to the amino acid, has never been considered seriously. More recently, experiments justify this attitude with the finding that isotopically labeled amino acids actually appear as such in the proteins of cells. Hence amino acids may be directly involved although it is still possible that they are first converted to some sort of derivative before condensation to protein. The necessary amino acids in the case of animals come directly from the diet and from the metabolic processes of the cells. The factors concerned in maintaining this supply have already been considered.

The second requirement for protein synthesis is the combination of amino acids with the formation of the peptide bond. This reaction is simple enough on the face of it but does not tend to go spontaneously. With a positive free-energy change of this magnitude, the equilibrium is far in the direction of hydrolysis, especially in view of the high water concentration customary in biological tissues. Since all proteins contain many peptide bonds, they are very unstable as a whole with respect to hydrolysis and can form only when some coupled system supplies the free energy needed to drive the condensation. Some workers in the field believe that less energy is needed to form each subsequent peptide bond as the chain gets longer. However, this possibility does not remove the free-energy requirement; it merely reduces it, and the basic problem is still unchanged.



$$ {}^{+}H_3N\overset{\overset{R}{|}}{C}HCOO^- + {}^{+}H_3N\overset{\overset{R'}{|}}{C}HCOO^- \leftrightharpoons {}^{+}H_3N\overset{\overset{R}{|}}{C}HCONH\overset{\overset{R'}{|}}{C}HCOO^- + H_2O \qquad \Delta F \simeq +3{,}000 \text{ cal.} $$

Obviously some system is needed to activate the amino acids or perhaps the growing peptide chain. One of the most interesting possibilities depends upon the formation of amino acid phosphates. These compounds can be made in the laboratory and are analogous to other high-energy phosphates. With these as the reactants, peptide bond formation would be highly favored and the free-energy change would be negative. This or a similar reaction was favored for a time because



$$^{+}H_3N\overset{R}{\overset{|}{C}}HCOO^- + ATP \rightleftharpoons {}^{+}H_3N\overset{R}{\overset{|}{C}}HCOPO_3H^- + ADP$$

$$\Big\downarrow\Big\uparrow \; {}^{+}H_3N\overset{R'}{\overset{|}{C}}HCOO^-$$

$$^{+}H_3N\overset{R}{\overset{|}{C}}HCONH\overset{R'}{\overset{|}{C}}HCOO^- + H_2PO_4^-$$

a dependence of peptide bond formation on ATP has been observed in studies on both simple peptides and proteins. However, diligent searches for amino acid phosphates have been to no avail. Hence these compounds may not be biologically important.

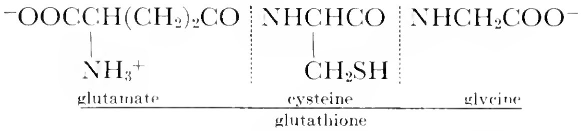
Work with isolated peptide syntheses has led to a different theory. The peptide glutathione occurs in most plant and animal cells and is believed to form in part as follows:

Enzyme + ATP ⇆ enzyme—PO_3H^- + ADP

Enzyme—PO_3H^- + glutamate ⇆ enzyme—glutamate + $H_2PO_4^-$

Enzyme—glutamate + amino acid → enzyme + peptide
(or peptide)

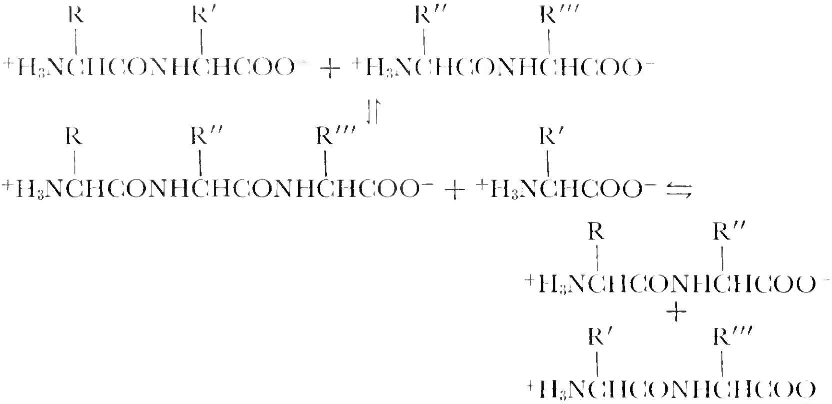
Although the reaction is rather well established for glutathione, this substance is not a peptide typical of proteins because one of the peptide bonds is γ rather than α.



$$^{-}OOC\underset{NH_3^+}{\underset{|}{C}}H(CH_2)_2CO \;\vdots\; NH\underset{CH_2SH}{\underset{|}{C}}HCO \;\vdots\; NHCH_2COO^-$$

Unfortunately there is yet no good evidence of this type of mechanism in protein synthesis. However, the following extension of these reactions has made them of greater interest.

Enzymes have been isolated that catalyze exchanges of amino acids between peptides and might allow utilization of glutathione and other peptides made as above. This process is called transpeptidation and may be represented as on page 488. Apparently a variety of similar



$$ {}^{+}H_3N\overset{R}{\overset{|}{C}}HCONH\overset{R'}{\overset{|}{C}}HCOO^- + {}^{+}H_3N\overset{R''}{\overset{|}{C}}HCONH\overset{R'''}{\overset{|}{C}}HCOO^- $$

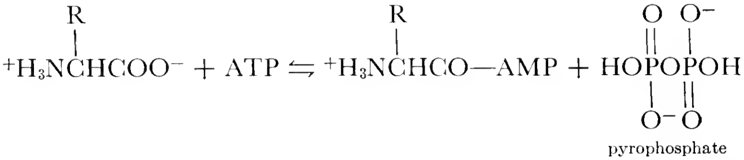
$$ \rightleftharpoons $$

$$ {}^{+}H_3N\overset{R}{\overset{|}{C}}HCONH\overset{R''}{\overset{|}{C}}HCONH\overset{R'''}{\overset{|}{C}}HCOO^- + {}^{+}H_3N\overset{R'}{\overset{|}{C}}HCOO^- \rightleftharpoons $$

$$ {}^{+}H_3N\overset{R}{\overset{|}{C}}HCONH\overset{R''}{\overset{|}{C}}HCOO^- + {}^{+}H_3N\overset{R'}{\overset{|}{C}}HCONH\overset{R'''}{\overset{|}{C}}HCOO $$

transfers occurs with the free energy of one peptide bond exchanged for that of another. By this means it is possible to conceive of a stepwise increase of molecular size at the expense of the ATP needed to form the original peptide bonds like those in glutathione. Again there is no direct experimental indication that such a process participates in protein synthesis.

More recently a second type of anhydride has been proposed for the activation of amino acids. ATP and other nucleotide phosphates can function in this process, where AMP represents the acyl form of



$$ {}^{+}H_3N\overset{R}{\overset{|}{C}}HCOO^- + ATP \rightleftharpoons {}^{+}H_3N\overset{R}{\overset{|}{C}}HCO\text{—}AMP + \underset{\text{pyrophosphate}}{HO\underset{\underset{O^-}{|}}{\overset{\overset{O}{\|}}{P}}O\underset{\underset{O}{\|}}{\overset{\overset{O^-}{|}}{P}}OH} $$

adenosine monophosphate (adenylic acid). These reactive anhydrides have been synthesized and polymerize spontaneously to yield polypeptides. Since a spontaneous reaction would be random, some system must be required to control specificity as discussed below.

Before leaving the energetics of peptide bonds, however, it is necessary to consider the stability of the completed bond. As pointed out earlier, the peptide bond is rather unstable with respect to hydrolysis, and at equilibrium there would be little peptide. To provide peptides and proteins for the purposes of cells, three possibilities appear to exist. First, hydrolysis is extensive but is compensated for by rapid and continuous synthesis. Since this procedure would necessitate the consumption of large quantities of ATP and other high-energy sources without recovery of this energy on hydrolysis, the inefficiency of such a system is unattractive. Furthermore, the energy demands of cells do not appear to be as high as would be required, and both peptides and enzymes are actually rather stable when dissolved in water for ordinary periods of time.

Second, since the peptide bond does resist simple hydrolysis on standing in water, a high energy of activation is indicated. This term refers to the phenomenon of energy needed merely to get a reaction started. Afterwards this energy reappears and is accompanied by the free-energy change characteristic of the reaction. Thus only very energetic molecules can begin to react, and the reaction does not start until energy is supplied to energize the molecules sufficiently. Therefore, a high energy of activation serves as a barrier even to otherwise spontaneous reactions. This factor probably allows one to keep proteins in the presence of water at ordinary temperatures.

Catalysts, however, lower energies of activation and reduce the molecular energy needed to initiate a reaction. When cells are broken open, such catalysts are found in the form of proteolytic enzymes. Therefore, inside cells the proteins must be protected from such enzymes. Perhaps this third factor in providing cells with proteins is achieved by confinement of the enzymes in some way or by storage in forms inactive in the cells but becoming active when the cells are ruptured. There is some evidence for both ideas. On the other hand, there may be some energy-coupling device in the cells which changes the protein enough to alter the free energy in the direction of stability and thus overcome even the effects of catalysts. Thus these or similar arrangements, perhaps in combination, are believed to ensure the necessary supply of cellular proteins.

Whatever the mechanism of formation of peptide bonds, it is quite clear that not all conceivable combinations of amino acids exist. Although there are many different proteins in an animal, the number is relatively small compared to the number of possibilities. Therefore, the sequences of the amino acids must be specifically controlled. Moreover, offspring have many proteins apparently identical with those of their parents. Hence reproduction must incorporate this control system into the developing individual. This need for a control mechanism is the third general requirement in protein biosynthesis.

Diverse studies suggest that the nucleic acids are involved in protein synthesis and help control protein structure. For example, when added substances interfere with the nucleic acids, protein synthesis is blocked. On the other hand, protein synthesis can be prevented under some conditions without immediately stopping the formation of nucleic acids. Thus it seems that the presence of the latter is required for protein synthesis, and some investigators feel that the ribose type of nucleic acid must actually be synthesized at the time the protein appears. Be that as it may, the consensus of opinion holds that both ribose and deoxyribonucleic acids are important factors. Since these macromolecules are strong acids, they undoubtedly occur in cells as anions, probably in the form of nucleoproteins.

During cell division (reproduction at the cellular level), the hereditary information is thought to reside in deoxyribonucleoproteins. Somehow this coded information induces the formation of specific protein material which in turn catalyzes the synthesis of deoxynucleoproteins like the originals. The two sets are then apportioned to each new daughter cell. The deoxyribonucleoprotein is postulated to function through the mediation of ribonucleoproteins which serve as intermediate patterns between the deoxyribonucleoprotein and the catalytic formation of the duplicate molecules.

In a similar way, the deoxyribonucleoproteins must exercise genetic control over the formation of all the new proteins necessary for the new cell. Theories have been advanced to account for the transfer of information from deoxyribonucleoprotein to ribonucleoprotein but are purely speculative up to now. There is some evidence suggesting a mechanism for the next step, control of protein structure by the ribonucleoprotein. The theory supposes the formation of activated intermediates of the nucleotide-amino acid type of page 488. From such compounds the amino acids are transferred to linkages on the ribonucleoprotein itself. When the required amino acids are available to fill the specific sites of the pattern ribonucleoprotein molecule, specific protein is formed.

Though this arrangement is the best so far devised, there are still some difficulties to be resolved. Ribonucleic acid contains only four different nucleotide components. Since at least twenty amino acids occur in most proteins, direct interaction governed only by the individual nucleotide components cannot account for the structural specificity of proteins. Furthermore, nucleic acids are believed to be rather rigid cylindrical molecules. If protein forms around the outside of this cylinder in direct contact with the pattern, spontaneous removal of the layer of finished protein becomes difficult. Then, too, once removed, the polypeptide chain must be specifically coiled to form a protein molecule with the required groups on the outside. This last difficulty becomes major for the globular proteins which occur in solution as spheres or ellipsoidal particles. Although all these problems of protein synthesis have been examined and suggestions made, no proposals are generally accepted and entirely free from serious objection.

Replacement of Proteins

When an animal is injured, the tissues involved must be repaired, primarily a matter of protein synthesis. In addition, the inherent thermodynamic instability of proteins in water implies that there may be some continuing loss of protein which must be made good. Although the biosynthetic mechanism of protein formation is difficult to establish, the replacement of proteins is easily shown.

Through studies with isotopes, it has been found that normal adult animals continually replace their body proteins. It appears that the various proteins are steadily decomposed and reassembled from the bodily supply of amino acids. The rates of these exchange processes vary with the type of protein and the animal species. Probably there are variations with the age of the individual, his diet, and pathological states as well.

For example, when radioactive leucine or tyrosine is fed to adult rats in nitrogen balance, radioactivity appears in the body proteins. Administration of labeled amino acid for a comparatively short time makes most radioactive those proteins which are being replaced most rapidly. Likewise, if rats fed the radioactive amino acids for a definite short time are then continued on diets with non-radioactive amino acids, the radioactivity is lost quickly from the proteins replaced rapidly and slowly from the more stable proteins. Studies have been made of these rates of appearance and disappearance of radioactivity from various proteins and tissues.

For instance, in rats the proteins of plasma, liver, and other internal organs are half-replaced every 6 to 7 days, a surprisingly short half-life. Even the skeletal proteins have exchange half-lives of only 21 days on the average. One would expect variations in these groups, but the important thing is that such studies indicate turnover of all proteins of animals, even the matrix materials of bone. Man with a longer life span also has longer half-lives for his proteins. Work with abnormal concentrations of stable isotopes leads to the following half-lives: 10 days for liver and serum proteins combined, and about 160 days for the combined proteins of brain, lung, skin, bone, and muscle (the last predominating in quantity).

Although it has been argued that the molecules affected by these exchanges are not broken completely down to amino acids, it is clear that such molecules must at least be partly degraded and then put back together. A more serious problem arises from reports that sections of various non-growing animal tissues in laboratory cultures do not incorporate radioactive amino acids into their proteins. This apparently contradictory finding can be reconciled by assuming major biochemical disturbances when the tissue is transferred from the animal. However, definite proof of such a hypothesis will be necessary to remove the doubts thus cast on the concept of protein turnover in intact animals. In this connection it may be mentioned that the exchange rates for the proteins of animals fed on low-nitrogen diets drop sharply. Apparently the animals have a mechanism for the conservation of proteins under these conditions. Protein breakdown does occur, of course, and the amino acids are slowly metabolized and lost.

Animals employ accelerated protein syntheses for replacement of proteins lost during starvation, wasting diseases, and mechanical tissue damage. Regeneration of liver is the classic example. When two-thirds of the liver is removed surgically from young rats, regeneration of the organ is complete in about 10 days in spite of the simultaneous repair of the other tissues injured in reaching the liver. Work of this sort raises the unsolved problem of termination of growth when the organ is regenerated or the wound healed. Why should the remaining liver tissue grow rapidly to its original size and shape and then stop abruptly? Cancer tissue also grows very rapidly but continues increasing. Perhaps it lacks the control system so mysteriously effective in normal tissues.

When animals are in nitrogen balance, protein synthesis and breakdown are equal. Isotopic studies have been made of the overall rates. In man the rate is 0.6 to 1.0 g. of protein per day per kg. of body weight. These values correspond to 40 to 70 g. daily for an adult male, a quantity equaling the recommended daily allowance of dietary protein. Dogs likewise synthesize about 0.6 g. of protein daily per kg. of body weight. Rats with shorter protein life make 2 g. daily per kg. of weight.

Plasma and Milk Proteins

Like all others, these extracellular proteins must be synthesized inside cells. In the case of the several plasma proteins, liver cells are the major but not the sole synthetic source. The fibrinogen important to blood clotting and serum albumin may be formed exclusively in the livers of man and the other animals. About 80 per cent of the globulin fraction also comes from liver with the remainder synthesized elsewhere. The γ-globulin group containing the antibodies arises from the widely distributed cells of the reticuloendothelial system. Once the proteins are formed inside cells, their method of secretion is still puzzling. Dialysis can hardly be a factor with molecules of such size.

The liver withdraws the essential amino acids from the blood and with a supply of the dietarily non-essential amino acids from the diet or metabolic reactions combines them to form the plasma proteins. When the diet provides all the essential components, the synthesis is rapid, and high levels of the plasma proteins are maintained. If the diet becomes markedly deficient in total nitrogen or any essential amino acid, protein synthesis drops quickly and the concentration of serum albumin falls. While somewhat affected, the globulin content of blood is more stable and fluctuates less extensively. Abnormalities, such as hepatitis and cirrhosis, affecting liver function produce similar results because the number of functioning cells is decreased.

With a large blood supply, the mammary glands of animals might use either the free amino acids or the serum proteins in the synthesis of milk proteins. The serum proteins could function rather directly with limited degradation and recombination into the new molecules. Or they could be split into the individual amino acids for a complete condensation process. In this last event, amino acids from the proteins would probably intermingle to some extent with those free in the blood.

At least a partial answer is available. Cows and goats use only free amino acids of the blood, and serum proteins do not participate except as they may be degraded to free amino acids outside the mammary gland. The so-called essential amino acids are incorporated as such. Glutamine, glutamic acid, and serine are also taken from the blood rather than being synthesized from other materials. It has not been established whether the remaining amino acids are supplied by the blood or are synthesized as needed.

Milk production represents protein synthesis on a large scale, especially among dairy cattle bred for this purpose. The scope and complexity of this operation explain in part the high nutritional requirements of lactating animals. Although milk differs with species, the variations are in the relative amounts rather than in the kinds of proteins. Hence there is no need to assume differences in mechanism.

Enzyme Synthesis

Partly because enzymes are relatively easy to determine quantitatively by studies of their catalytic activities, much work has been done on enzyme synthesis. In general the earlier remarks on the biosynthesis of proteins apply equally well to enzymes. Nucleic acids are definitely involved, amino acids must be supplied, and energy must be coupled to the process. In addition, there are some special features of interest.

Microorganisms containing little or none of a particular enzyme can synthesize a rather large quantity of that enzyme when the environment is changed. Although similar experiments with animals are more difficult, animals seem to possess at least a limited ability to increase the concentrations of enzymes, thus adapting themselves to changing situations. The usual requirements for protein synthesis must be met plus an additional need for a stimulating factor called the inducer. Since the inducer is essential for the synthesis of the new enzymatic material, the whole process is called induced or adaptive enzyme synthesis.

The inducers are substrates of the enzyme, products of its catalytic activity, or compounds closely related chemically to the substrates or products. Though not all substrates induce enzyme synthesis, many do. This arrangement makes it unnecessary for a cell to contain a full measure of all the enzymes it can make. Rather the cell relies on an induced synthesis of enzymes to adapt it to new environments and thereby conserves the supply of amino acids. Much study of this phenomenon has supported and extended the observations made with the non-enzymatic proteins. Perhaps formation of this latter group also depends on the presence of inducers which may be normal cell constituents. Although extensively studied, the role of the inducer has proved elusive. The several theories are in conflict with each other and with portions of the experimental evidence. Nevertheless, study is continuing on this mechanism, for many workers believe it may provide the clue to the whole problem of protein synthesis.

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Energy

21

All living things withdraw energy from their environments and use it for their various physiological operations, including growth, movement, chemical transformations, temperature control, and luminescence. Thus cells are always at the mercy of their environments in this fundamental regard. Without energy there can be no life. However, evolution has led to the development of species capable of utilizing different forms of energy. At least indirectly all life depends on heat, primarily in the range between the freezing and boiling points of water. Most species are limited to narrow parts of this range and have quite definite optimum environmental temperatures. All plants and the great group of cold-blooded animals possess internal temperatures approximating the external temperature. Such organisms cannot function when the environmental temperature drops below characteristic levels. Not all die, of course, since the species of temperate and cold climates become dormant or possess reproductive stages that are cold resistant and do not complete the life cycle until the return of warm weather.

Mammals and birds have evolved with mechanisms for the control and maintenance of their internal temperatures. These specialized forms can thus supply their own needed heat energy, providing that a suitable supply of chemical energy is available in their food materials. Many plants and microorganisms have similar requirements for such chemical energy, but some classed as photosynthetic species do not. This last group is adapted for the absorption of radiant energy and its conversion to necessary chemical energy.

Since the energy needed in physiological processes is ultimately converted to heat, most of it is dissipated slowly to the environment. As already noted, many cellular reactions require energy, and of these a number require amounts far beyond the energies available at the level of heat within the biological range of temperatures. Thus animals must assimilate materials of high chemical energy and degrade them by processes utilizing this energy both chemically and thermally. Therefore, animals depend directly or indirectly upon the photosynthetic plants which convert radiation to chemical energy in forms that animals can use. The meat-eating animals are, of course, dependent on the herbivores and through them on the green plants. In this sense all animals are parasitic and could not survive by themselves on an earth without plants.

ENERGY CONTENTS OF FOODS

Although inorganic materials are necessary to them, animals cannot recover chemical energy from these materials. Water, carbon dioxide, and minerals are important in animal metabolism but as solvent, reactants, catalysts, and structural materials. Organic compounds then must serve as the energy sources, falling into the three major food classes, carbohydrates, lipides, and proteins with their related compounds. The metabolic reactions undergone by these materials have already been discussed. Some of the energy-yielding steps have been mentioned also, but neither the overall energies of foodstuffs nor the total needs of animals have been considered.

First there is one point of theoretical importance worthy of mention. As noted above, animals must have chemical energy, usually referred to as available free energy. However, free-energy changes are not easily measured when complex systems like animals are involved. Therefore, heat contents are measured instead, usually by calorimetric procedures, although heat content and free energy are not the same thing.

The thermodynamic equation

$$\Delta F = \Delta H - T\,\Delta S$$

compares the change in free energy ΔF with the change in total heat content ΔH for any process. T is the absolute temperature and ΔS the change in entropy, which is a measure of disorder or randomness in the system. From this equation it is clear that free energy and heat changes may differ markedly, and in fact they do in many chemical reactions. Fortunately, in the overall metabolic processes of animals,

$T\,\Delta S$ is often fairly small relative to ΔF and ΔH and is neglected, because neither ΔF nor ΔS can be estimated in most cases. For the complete reaction

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

$\Delta F = -686{,}000$ cal./mole and $\Delta H = -674{,}000$ cal./mole, leaving $T\,\Delta S = 12{,}000$ cal./mole, a relatively small factor. Similar situations occur for other overall oxidations but not for some of the possible intermediate reactions like

$$CH_3COOPO_3H^- + H_2O \rightarrow CH_3COO^- + H_2PO_4^-$$

In this case $\Delta F = -13{,}500$ cal./mole, $\Delta H = -7{,}200$ cal./mole, and $T\,\Delta S = 6{,}300$ cal./mole, so $T\,\Delta S$ can hardly be neglected in this case. Such reactions as this one must be avoided by efficient cells since the loss in the $T\,\Delta S$ is irretrievable. Metabolic pathways probably do not involve simple hydrolyses of high-energy compounds like acetyl phosphate. Rather, coupled reactions transfer most of the available free energy to some other useful intermediate.

Heats of Combustion

Although the chemical energy and heat content of foods differ, only measurements of the latter are common. They are often made with a bomb calorimeter, a device providing for the oxidation of the organic materials of foods to carbon dioxide, water, and nitrogen. Provision is made for the determination of the heat evolved during the oxidation.

The bomb itself is a heavy steel container in which a weighed sample of the food material is ignited in an atmosphere of oxygen under pressure. The ignition is achieved electrically by passing a known current for a known length of time between the electrodes of an arc or through a wire filament. Heat from this combustion process raises the temperature of the bomb and an insulated water bath in which the bomb is supported. Since the temperature change produced by a given amount of heat is known, the total heat evolved inside the bomb is readily calculated. This result must be corrected for any losses from the bath to the room and for the heat resulting from the known amount of electricity used in the ignition. The values after adjustment are called heats of combustion and are expressed in calories per gram of material burned. A calorie is the heat needed to raise the temperature of 1 g. of water from 15 to 16°C. Since this is a rather small quantity of heat, heats of combustion are often expressed in kilocalories (kcal.) equaling 1,000 small calories. Many books on nutrition and most popular literature refer to the "large" calorie, written as Calorie or Cal., which is the kilocalorie.

Animal Calorimetry

Use of the bomb calorimeter provides data on the complete combustion of foodstuffs. However, animals may be unable to oxidize the material so completely. As a matter of fact in Chapter 20 we have seen that animals excrete their waste nitrogen in reduced forms like ammonia, urea, uric acid, allantoin, amino acids, and peptides. Therefore, calorimetric methods using animals have been developed to ascertain the heat actually available to the animal.

Direct calorimetry with animals is similar in principle to work with the bomb calorimeter but with refinements designed to provide an environment that ensures a normal metabolism. For this purpose the temperature must be controlled to a suitable range for the species concerned and air must be provided. Then the animal burns the food metabolically in contrast to the purely chemical processes in the bomb calorimeter.

Since the animal generates heat, the insulated chamber would become abnormally hot without provision for cooling. The best arrangement uses a system of water pipes to keep the compartment at constant temperature. Measurements of the inflowing and outflowing water temperatures and of the rate of flow allow calculation of the heat loss by the animal by radiation and conduction. The other major loss of heat is in the evaporation of water in the expired air or as perspiration. This factor is determined by trapping and weighing the water vapor in the air pumped out of the calorimeter and calculating the heat needed to evaporate the observed quantity of water. About 0.58 kcal. is necessary per g. of water at 25°C. with the value changing somewhat with temperature. Heat lost by evaporation is about 20 to 25 per cent of the total for common mammals, so this transfer mechanism is important.

The amount of heat evolved by an animal depends upon the species, size, individual, environmental temperature, and activity. This heat must come from the chemical energy of the diet. Therefore, any change in the output of heat is normally reflected in a change in the diet, either in composition or quantity or both. The interrelationships with heat production can all be studied with calorimeters. Such work has shown that different classes of foods yield different quantities of energy as shown in Table 21–1.

Since digestion is never completely efficient, some of the potentially available energy is lost. One column of the table is corrected for this

TABLE 21–1. Approximate Heats of Combustion of Classes of Nutrients

Nutrient	Heats of Combustion, kcal./g. Direct Animal Calorimetry	Corrected for Digestion Losses	Bomb Calorimetry
Carbohydrates	4	4.1	4.1
Fats	9	9.5	9.5
Proteins	4	4.4	5.6

factor, taking 98, 95, and 92 per cent as the respective digestive efficiencies of carbohydrates, fats, and proteins. These percentages certainly vary with conditions but are taken as common values for the dietary materials that are available to the animal in question. Cellulose, for example, is not considered in studies with man.

When the corrected data for animals are compared with results from the bomb, it is apparent that carbohydrates and fats are completely oxidized. Proteins, on the other hand, are not, as shown by the larger value obtained in the bomb. The difference is attributed to the necessity for excreting nitrogen in reduced forms. The relatively high heats of combustion of fats are correlated with their low level of oxidation. Fats are the lowest in oxygen of the common dietary materials, hence are the least oxidized to start with and yield the most energy on oxidation. This high energy content especially suits fats for their role in energy storage. All the values tabulated are averages since the individual compounds differ somewhat. For example,

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad \Delta H = -3.75 \text{ kcal./g.}$$

$$\underset{\text{starch}}{(C_6H_{10}O_5)_X} + 6XO_2 \rightarrow 6XCO_2 + 5XH_2O \qquad \Delta H = -4.2 \text{ kcal./g.}$$

where X is the number of hexose units per starch molecule. The difference in the ΔH values in these cases is attributed to the removal of water when hexose units are joined to form starch. This process requires energy which is partly recovered as heat during the burning or metabolism of starch. Therefore, the heats of combustion reported can be taken only as representative of the classes of materials. The observed range seems to be within a few tenths kilocalorie of the figures selected.

Because direct calorimetry with a large animal severely restricts the animal and requires complex and expensive calorimeters, indirect methods have been sought. All these methods are based on the fact that there is a close relationship between the heat produced and the weights of the materials concerned. In the oxidation of glucose as an example, 6 moles of oxygen is required and 6 moles of carbon dioxide formed. Therefore, a determination of either gas will be a measure of the quantity of glucose metabolized when this sugar is fed to an animal, and the heat produced can be calculated at the rate of 3.75 kcal./g. of glucose. More highly reduced foods involve different proportions of oxygen and carbon dioxide, but the calculation is the same in principle. Since most natural food materials are mixtures of classes of foods, it is necessary to measure both oxygen and carbon dioxide in order to estimate the relative extents to which these classes are contributing energy. The general procedure is considered in more detail on pages 504 to 506.

Direct calorimetry with known foods permits a comparison of the heat actually obtained and that expected after correction for digestive and excretory losses. Any differences indicate that food intake and heat production are out of balance. When the observed heat is lower than the anticipated value, the animal is not oxidizing the food completely. Either it is not digested with the usual efficiency, or free energy is being conserved and not converted to heat. This latter situation is common during the growth of young animals, recovery after injury or illness, or the deposition of fat. Whenever the heat measured exceeds that expected, the food ingested must be inadequate in quantity or composition, requiring the animal to draw on its reserves and metabolize body materials with a loss in weight. Likewise, indirect calorimetry with foods of known composition reveals differences between observed and expected oxygen consumption and carbon dioxide evolution. Such results indicate gain or loss in weight.

For the initial study of the heat contents of foods by animal calorimetry, the animal must be in metabolic equilibrium with a constant body weight. When rations are studied under these conditions, values like those of Table 21–2 are obtained for the heat contents, called caloric values in most nutrition tables. Data of this type are used to compile diets meeting the levels of energy and nutrients required by particular individuals. The Appendix contains a more extensive table of the same sort. A comparison of values from the two tables for given foods reveals numerous differences. For one thing these variations reflect the inhomogeneity of most foods. Differences in experimental techniques, efficiency of digestion, and differences between individuals account for some of the variations. As a result, the values reported can be considered only as approximations and used as such.

TABLE 21–2. Caloric Values and Compositions of Some Human Foods

Food	Caloric Value, kcal. g.	Composition, %: Carbohydrate	Fat	Protein	Water	Ash	Crude Fiber
Beef, fat	3.0	Trace	25	19	55	0.9	. . .
Beef, lean	1.2	Trace	4	22	74	1.0	. . .
Halibut	1.3	Trace	6	18	75	1.0	. . .
Eggs	1.5	0.6	11	14	74	0.9	. . .
Whole milk	0.6	4.7	3.5	3.4	88	0.8	. . .
Butter	7.9	0.5	85	0.8	14	0.2	. . .
Cottage cheese	1.7	3.0	2	38	52	4.4	. . .
Bread	2.2	54	0.8	6	37	1.2	0.8
Rice	3.3	17	0.5	8	13	0.8	0.5
Peanuts	6.0	24	44	27	3	2	. . .
Apples	0.4	14	Trace	0.4	84	0.4	1.3
Tomatoes	0.2	4	Trace	1	93	0.6	0.8
Potatoes	0.7	21	0.1	2	75	1.1	0.7
Peas (dried)	2.9	52	2	23	14	3	5.6
Honey	3.0	80	0	0.3	19	0.3	. . .
Chocolate	4.7	60	25	2	12	1.0	. . .

Since the heat contents of carbohydrates, fats, and proteins are similar within each class and average values are known (page 499), the simplest procedure for evaluating the heat content of a food is calculation from composition data. The carbohydrate, fat, and protein contents of the food are determined by standard methods, and the total heat content calculated from the heat contents of these three classes of constituents. It is assumed that only these materials contain appreciable quantities of energy. Direct calorimetry indicates that this assumption seems to be generally valid. The final calculation can be written in equation form.

$$\text{Heat content (kcal./g.)} = 0.04\,(\%\ \text{carbohydrate} + \%\ \text{protein}) + 0.09\,(\%\ \text{fat})$$

ENERGY FROM FOODS

Practically all the energy of animals comes from their foods via the metabolic reactions. In some cases the food is oxidized rather directly and as completely as the pathways of metabolism allow. In other cases components of the food are stored for later use as discussed elsewhere. The general food requirements of individual animals are determined by their overall energy requirements which depend upon the several factors considered below.

Digestibility

The tabulated heat contents are based on the assumption that carbohydrates are completely digestible except for a small inefficiency factor of about 2 per cent. However, many mammals can digest only starches of the polysaccharide group. Therefore, cellulose, inulin, and pentosans must be excluded from consideration for these animals. Others, including the ruminants especially, digest and utilize a portion of these other polysaccharides. Thus the digestive differences of animals lead to differences in the energies recovered from ingested foods.

In Table 21–3 the column headed "Crude Fiber" refers to material (mostly cellulose) not utilized by some species but partly digested by others. Many plant materials, like leaves, stems, stalks, and twigs, supply little energy to fowl, human beings, pigs, rats, dogs, and the like. Since digestion of crude fiber is never complete for any animal, the energy contents of rations for ruminants must be determined for each species. Furthermore, the fraction of the crude fiber digested depends upon the nature of the ration. Hence feeding trials must be conducted with each ration of interest.

TABLE 21–3. Digestibility of a Mixed Ration of Alfalfa and Cornmeal for Steers

Nutrient Distribution	Nitrogen-free Extract	Crude Fiber	Crude Protein	Ether Extract
Nutrients ingested, g.	1140	340	247	51
Nutrients excreted in feces, g.	115	220	71	18
Nutrients digested, g.	1030	120	176	33
Digestion coefficient, %	90	35	71	65

Results from studies of this type are often expressed as total digestible nutrients based on the classes of nutrients present and the extent to which they are digested in the experiment. The estimation of digestible nutrients is really a balance study in which the nutrients consumed are compared with the amounts of these nutrients appearing in the feces. The amount of each nutrient excreted is subtracted from that present in the feed, and the remainder is recorded as nutrient digested. The percentage of the nutrient digested is known as the digestion coefficient. Digestion coefficients are usually determined for crude protein, crude fiber (cellulose), nitrogen-free extract (starches and sugars), and ether extract (fats). Before a digestion trial can be conducted, it is necessary that the diet be fed in constant amounts to the experimental subject for a preliminary period long enough to ensure that the daily fecal output is representative of the daily food intake. Feces are collected for a predetermined experimental period in which records are kept of both food consumed and nutrients excreted in the feces.

Table 21–4 displays the results of a typical digestion trial. Note that the digestibility of the material in the ether-extract fraction is below the figure taken on page 499 as the digestive efficiency of fats in human beings. Part of this difference may be correlated with species, but most of it is probably due to inclusion of steroids and other complex lipides not digested. In a similar way the digestion coefficient of protein also appears low because indigestible nitrogen compounds like the porphyrins are included since crude protein is based on the determination of the total nitrogen. This value is then multiplied by the factor 6.25 for conversion to crude protein. The nitrogen-free extract is determined by extracting the feed with dilute acid and alkali with a standard period of heating. Cellulose and lignin are insoluble and make up the crude fiber. The soluble extracts are titrated for reducing sugars and the results expressed as glucose. This procedure measures sugars and the readily hydrolyzed polysaccharides like starch, inulin, hemicellulose, and pentosans. All are at least partly digested by ruminants and horses, but the last three are not

TABLE 21–4. Total Digestible Nutrients in 100 Pounds of a Steer Ration

Nutrient	Quantity, lb.	Digestion Coefficient, %	Digested Portion, lb.
Nitrogen-free extract	60	90	54
Crude fiber	16	35	6
Crude protein	11	71	8
Ether extract*	2.3 (× 2.25)	65	3
Total digestible nutrients			71

* The ether extract is multiplied by the factor 2.25 ($\frac{9}{4}$) to place all the nutrients on the same caloric basis, thus adjusting for the high yield of energy from fats.

utilized by most other animals. Actually it is surprising that the digestion coefficients are as high as those tabulated when the assumptions and inaccuracies are considered.

When an animal feeder wishes to calculate the total digestible nutrients of a given feed or mixture of feeds, he merely multiplies the amount of such nutrient in 100 lb. of ration by its digestion coefficient and then combines these values to obtain the total amount of nutrients available to the animal organism, as indicated in Table 21–4. The value so obtained is a useful index of the caloric value of a feed when the digestion coefficients are known for the ration and the animal concerned. Differences between species have already been discussed. In addition, the digestibility of one feed component may be affected by the presence and amounts of other components. The level of feed intake also affects the results. Moreover, losses in methane and urine may amount to 15 per cent of the heat content of the ration and are ignored by this method of calculation. Nevertheless, in feeding practice wide use has been made of the total digestible nutrients in comparing rations and their components for feeding domestic animals.

Respiratory Quotients

Indirect calorimetry with animals is based in part on measurements of oxygen consumed and carbon dioxide evolved. Oxygen uptake is determined by calculation from the change in either the volume or the pressure that occurs when oxygen is utilized. Since carbon dioxide is expelled at the same time, a correction is made after a separate determination of the carbon dioxide. Or this latter gas is continuously absorbed and an effect on pressure thus avoided. The carbon dioxide is determined either by absorption with calcium oxide and weighed or by absorption in potassium hydroxide solution from which it is liberated with an excess of acid and its volume measured. Respiration studies involve chambers for animals and man or face masks for human beings with provision for sampling of the inspired and expired gases.

The proportions of oxygen and carbon dioxide vary with the nature of the materials metabolized as revealed in the following balanced reactions:

$$\underset{\text{starch}}{(C_6H_{10}O_5)_X} + 6XO_2 \rightarrow 6XCO_2 + 5XH_2O + \text{heat}$$

$$\underset{\text{tripalmitin}}{C_{51}H_{98}O_6} + 72.5O_2 \rightarrow 51CO_2 + 49H_2O + \text{heat}$$

For starch the ratio of carbon dioxide produced to oxygen taken up is 6X : 6X (1.0), while the ratio for the fat is 51 : 72.5 (0.70). Thus these ratios are characteristic of the type of metabolite and suggest the nature of material being utilized. They are called respiratory quotients and are calculated from balanced reactions or from actual measurements of the gases.

Although the respiratory quotients differ somewhat for the different members of each class of nutrient, the range is rather small. Ordinary carbohydrates are all rather close to 1.0, fats to 0.7, and proteins to 0.8. Since standard diets are mixtures of all three, the respiratory quotients observed with normal animals are averages weighted for dietary composition. Special conditions lead to characteristic respiratory quotients.

Fasting animals quickly mobilize and use up their carbohydrate reserves. Thereafter the respiratory quotient is approximately 0.7. If starvation is sufficiently prolonged, the depot fat will be depleted. Use of body protein increases and the respiratory quotient rises somewhat. On the other hand, heavy intakes of carbohydrate lead to high respiratory quotients as carbohydrate is converted to fat and stored. This procedure diminishes oxygen uptake and increases the relative production of carbon dioxide as some carbon atoms are reduced by oxidation of others. The formation of palmitic acid illustrates the point when written as follows:

$$\underset{\text{glucose}}{5C_6H_{12}O_6} + 7O_2 \rightarrow \underset{\text{palmitic acid}}{C_{16}H_{32}O_2} + 14CO_2 + 14H_2O$$

with a respiratory quotient of 2.0. Values of this sort have been observed in hogs fed a mixture of starch and glucose.

Low respiratory quotients occur in diabetes. This disease causes extensive excretion of glucose and ketones, and formation of the latter represents incomplete degradation of fats to carbon dioxide. The effect may be illustrated with palmitic acid. The carbon dioxide to

$$\underset{\text{palmitic acid}}{C_{16}H_{32}O_2} + 19O_2 \rightarrow \underset{\text{acetoacetic acid}}{CH_3COCH_2COOH} + 12CO_2 + 13H_2O$$

oxygen ratio in this case is 0.63, a value below that normally observed. Thus it appears that respiratory quotients can reflect the dietary or metabolic state of an animal. However, exact statements cannot be made because diets are complex mixtures and because the relative activities of the various pathways of the metabolic network are unknown. As a result, representative balanced reactions cannot be chosen to summarize the results of metabolism.

Oxygen consumption may be correlated in another way with metab-

TABLE 21–5. Oxygen Uptake and Heat Content of Nutrients

	Carbohydrate	Fat	Protein
Heat content, kcal./g. digested	4.2	9.4	4.3
Oxygen uptake, liters/g. digested	0.83	2.02	0.97
Ratio, kcal./liter of oxygen	5.0	4.7	4.5

olism. Using average values, the relationships of Table 21–5 show a degree of constancy for the heat produced per unit of oxygen when the different classes of nutrients are completely oxidized. This dependence permits the estimation of heat of reaction from measurement of the oxygen uptake.

Energy Balance

Growing animals ingest food sufficient to supply energy for the synthetic purposes of growth, for mechanical activity, and for replacement of heat lost. Adults still need energy for the synthesis of needed repair and replacement materials but do not need energy for growth. Most of the energy requirements of mature animals probably serve to satisfy activity and heat losses. Of these two factors, the first depends upon the size and movement of the animal and is considered in the later section on muscular activity. Loss of heat depends upon individual and species variations in surface area, sweat glands and their activity, blood capillaries in the skin, rates of respiration and heart beat, nervous state, excretion of waste products, physical activity, and insulating materials like hair, fur, and feathers. Heat loss also depends on environmental factors like temperature, humidity, air movement, contact with heat conductors, and artificial insulators like clothing.

With such complex variations the heat losses of individuals of normal size fluctuate widely for any given species. Human beings lose heat to their environments by the means and in the amounts shown.

Process	Heat Loss, kcal./day
Warming expired air	40–60
Excretion of urine and feces at body temperature	70–90
Evaporation of water from lungs and skin	300–1,200
Convection from body surface	500–1,500
Radiation from body surface	500–1,500

Clearly the last three factors are by far the most important except perhaps in hot moist climates where all losses may be reduced well below the lowest levels shown. For example, radiation loses its importance when prolonged high air temperatures raise the temperature of the surfaces of the environment above that of the body (37°C.). Under such conditions of hot days and nights, the body may actually gain a net amount of heat over at least part of the day.

In spite of all the factors modifying heat loss, all mammals and birds have temperature-control mechanisms which compensate effectively for large environmental and physiological changes. Temperature regulation permits a much broader scope of action to animals thus equipped, enabling them to survive and function more or less normally when weather conditions are extreme. Plants, insects, and cold-blooded animals must be sheltered, become dormant, or die when the environmental temperature becomes exceptionally hot or cold.

Animals normally lose heat at characteristic rates even when at rest. This heat is replaced as a result of metabolism with some arising from the continuous muscular work of the heart beat, peristalsis, and respiration. The combined metabolic and physical processes occurring in fasting animals at rest at 20 to 25°C. are called the basal metabolism and represent heat losses under the special conditions mentioned.

Basal Metabolism

Measurements of heat loss can be made under conditions avoiding or standardizing many of the physiological and environmental factors mentioned above. Even though species and individuals still differ, some of these differences can be correlated with special physiological properties of both scientific and medical value.

For study of the basal metabolism (basal metabolic rate), identical conditions must be used for all individuals examined in order to obtain comparable results. Human beings are studied in all modern hospitals after a night's sleep without food and after 30 min. of complete rest and quiet. Under these standardized conditions, heat production is calculated from the rates of oxygen consumption and carbon dioxide evolution. This basal metabolism depends upon various factors like age, sex, body surface area, diet, levels of certain hormones (especially thyroid hormones), environmental temperature, and to some extent the type of daily activity.

Body area reflects both height and weight and shows a remarkably close correlation with the basal metabolic rates of the warm-blooded

TABLE 21–6. Basal Heat Production by Some Mammals and Birds

Species	Body Weight, kg.	Basal Metabolism, kcal./kg./day	Basal Metabolism per Unit of Surface, kcal./m.^{2}/day
Horse	441	11	950
Pig	128	19	1,080
Man	64	32	1,040
Dog	15	52	1,040
Goose	3.5	67	970
Chicken	2.0	71	940
Mouse	0.018	75	920

animals. (See Table 21–6.) Since body area can account for large differences in basal metabolism, it is usually corrected for by expressing results in kilocalories per square meter per day. Although body area is comparatively difficult to measure, it can be calculated from body dimensions, using a special equation for each species. For man the relationship is

$$A = W^{0.425} \times H^{0.725} \times 7.184 \times 10^{-3}$$

where A is area in square meters, W is weight in kilograms, and H is height in centimeters.

After taking the body area into consideration, the effects of both sex and age are evident for human beings in Table 21–7. Males have slightly higher rates for basal metabolism, and there is a continual decrease with increasing age. Additional factors modifying the basal

TABLE 21–7. The Effect of Sex and Age on the Basal Metabolism of Human Beings

Age, years	Average Metabolism, kcal./m.^{2}/hr. Males	Females
5	53	52
10	50	46
15	45	40
20	41	37
30	40	36
40	38	35
60	36	34
75	34	32

TABLE 21–8. Conditions Altering the Basal Metabolism of Human Beings from the Normal for Given Sex, Age, and Body Area

Depressing	Elevating
Sleep	Adaptation to strenuous daily activity
Malnutrition including starvation	Pregnancy (latter half)
Hypothyroidism	Hyperthyroidism
Pituitary deficiencies	Fever
Addison's disease	Leukemia
Anesthetics and certain other drugs	Stimulatory drugs like caffeine, epinephrine, benzedrine
Tropical climates	Frigid environments

metabolic rate are classified qualitatively in Table 21–8. From these lists the diagnostic usefulness of such metabolic studies becomes clear in the identification of certain pathological states. A number of less-well-known diseases also raise or depress the basal metabolic rate.

Muscular Activity

The biochemical processes of movement require energy which is taken in the form of chemical energy from the diet. Making the somewhat inexact assumption that changes in free energy and heat content are equal, the energy for muscular activity is added to that lost as heat in arriving at the total energy of metabolism. Table 21–9 contains estimates of the energy required by a man of about 70 kg. at different levels of activity. The energy for sleeping and resting is consumed in changing body position, for muscle tremors and twitches,

TABLE 21–9. Muscular Activity and the Energy Expenditure of Men

Activity	Energy Requirement, kcal./hr.
Sleeping	65
Awake, at rest	77
Sitting, at rest	100
Reading aloud	105
Standing relaxed	105
Standing at attention	115
Light exercise	170
Active exercise	290
Severe exercise	450
Exercise at the physical limit	600

and in the muscles of the heart, intestinal, and respiratory systems. Since little of this energy produces physical displacements of either the individual or parts of the environment, most of it is converted to heat and lost as such. The increments beyond this minimum level of activity are used to overcome gravity, move from place to place, or transport objects. Again much of the energy appears as body heat but some is converted into potential energy of position.

Intense emotion may increase the expenditure of energy by 5 to 10 per cent above the basal level, a relatively small contribution when compared with exercise. Profound mental activity has an even lower energy requirement, making it unnecessary to allot anything in the diet as food for thought.

Turning to the operation of muscle, agreement has not been reached on the mechanism of muscle action, although some general requirements are well established. In the first place, high-energy phosphates participate as intermediates in the transfer of chemical energy from metabolites to muscle. A limited supply of creatine phosphate (phosphocreatine) acts as an energy reserve, making available the adenosine triphosphate (ATP) which functions as the actual energy carrier. The creatine phosphate is formed by energy transfers, starting with glycogen. Thus muscle glycogen becomes the major starting point for muscular energy.

Nerve impulses induce muscular contraction by splitting ATP into ADP and phosphate and converting the chemical energy thus made available into mechanical energy. The exact nature and sequence of events are still under debate. The higher animals are about 40 per cent muscle, which in turn is mainly water and protein. Some of the proteins are dissolved in the cellular fluid, while two others called actin and myosin make up the contractile fibers. Myosin and actin comprise respectively about 38 per cent and 14 per cent of the proteins of rabbit muscle.

One theory postulates that contraction involves the shortening of actomyosin, which is a complex of about 3 parts of myosin with 1 of actin. Since muscular contraction is very rapid, fast reactions are required and these are visualized as ionic changes in electrical potential near the surface of the actomyosin complex. As a result, attractions between unlike charges on the actomyosin cause a shortening of the complex which is muscle contraction. This process is represented schematically in Figures 13–1 and 13–2 with the fixed charges of the protein surrounded by ion atmospheres shown as ions of ATP and potassium.

It is proposed that stimulation by a nerve alters the ion atmosphere enough to allow the electrostatic attraction suggested. Contraction is accompanied by liberation of potassium ions which then can be dialyzed out. In addition, ATP is split but whether before or after the contraction is not yet known. The cleavage of ATP is attributed tentatively to the myosin portion of the protein complex, since myosin is an active phosphatase for this substrate. Some workers view the cleavage as following contraction and supplying energy to charge the system preparatory to another contraction. Others feel that ATP is split just before contraction, and the resulting energy causes the changes in the distribution of ions followed by contraction of the protein complex. In any case, the energy is efficiently transferred.

Because the concentration of ATP in muscle is low at any given time and even the reserve of creatine phosphate is limited, prolonged muscular activity depends upon metabolism to provide a continuing supply of energy. Oxygen is transported by the hemoglobin of the blood and transferred to a related protein in muscle called myoglobin. Then a demand for ATP leads to the metabolic breakdown of glycogen to carbon dioxide and water with the energy passed to ATP for use in muscle contraction. The reactions include those of glycolysis (page 168) or the pentosephosphate pathway (page 223), the tricarboxylic acid cycle (page 171), and the electron-transfer system (page 172).

Sometimes, however, emergency requirements for energy temporarily exceed the rates of respiration and circulation, and the supply of oxygen becomes inadequate. In this event, glycogen is metabolized via glycolysis only. For this purpose DPN^+ must be available, but electrons cannot be transferred through flavoproteins and cytochromes without oxygen. Therefore, some other oxidation of DPNH must occur. In muscle this reaction involves pyruvate, which is reduced to lactate to give the DPN^+ back again.

$$CH_3COCOO^- + DPNH + H_3O^+ \xleftrightarrow[\text{dehydrogenase}]{\text{lactic}} CH_3CHOHCOO^- + DPN^+ + H_2O$$

Lactate accumulates in working muscle until increased respiration and circulation provide an adequate supply of oxygen for complete metabolic oxidation. Some lactate dialyzes into the blood and is metabolized in other parts of the body. When physical activity ceases, part of the lactate reverts through the glycolytic system to glycogen. The proportion thus returning to storage depends upon the magnitude and extent of the exertion undergone. These processes are summarized in Figure 21–1. The creatine phosphate thus acts to stabilize

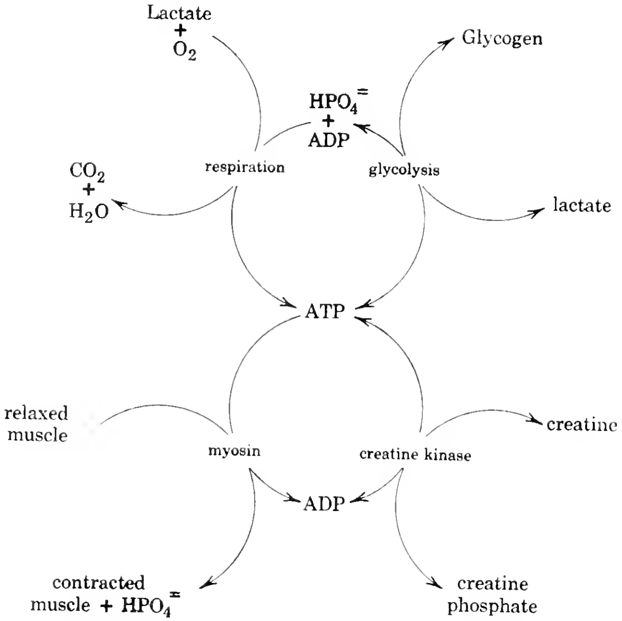
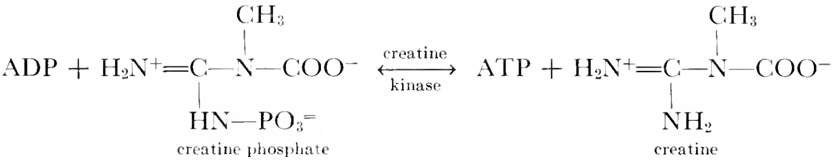


FIGURE 21-1. The overall energy transfers in muscle contraction.

the supply of ATP for proper muscle function and operates through the reversible reaction



$$\mathrm{ADP} + \mathrm{H_2N^+{=}}\underset{\substack{|\\ \mathrm{HN{-}PO_3^{=}}}}{\mathrm{C}}\mathrm{{-}}\overset{\substack{\mathrm{CH_3}\\ |}}{\mathrm{N}}\mathrm{{-}COO^-} \xleftrightarrow[\text{kinase}]{\text{creatine}} \mathrm{ATP} + \mathrm{H_2N^+{=}}\underset{\substack{|\\ \mathrm{NH_2}}}{\mathrm{C}}\mathrm{{-}}\overset{\substack{\mathrm{CH_3}\\ |}}{\mathrm{N}}\mathrm{{-}COO^-}$$

creatine phosphate — creatine

Energy Allowances

As was indicated in Table 21–9, the energy needs depend upon the muscular activity as well as on the size, age, sex, etc., of animals. Human beings at work expend energy at the rates shown in the table with each of their occupations corresponding to one of the various degrees of exercise. Average values for people in different types of jobs are shown in Table 21–10. These allowances must be adjusted for individual differences according to the principles outlined earlier. See the Appendix for additional data. Activities similar to these in level of exertion have similar energy allowances. Note that the basal metabolic rate depends on daily muscular activity to some extent as

TABLE 21–10. Energy Allowances for Persons in Different Occupations
(In Kilocalories per Day)

Type of Work	Daily Allowance	Basal Metabolism	Work Increment
Office work	2,600	1,990	600
Carpenter, painter	3,300	1,980	1,300
Mason	3,800	2,050	1,700
Logger	5,600	2,240	3,400

mentioned on page 507. The increments allotted for work must be large when the work is heavy.

Although it would be more accurate perhaps to report energy allowances for domestic animals in terms of the available food energy, individual and species variations and specific dietary effects make this difficult. As a practical compromise, it is customary to use digestible nutrients as the index of energy content and to set daily allowances in these terms. Recommended levels of digestible nutrients are listed in the Appendix for beef and dairy cattle, sheep, horses, and swine. Tables of the digestible nutrients of various stock feeds are also included.

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Animal Nutrition, fourth edition. L. A. Maynard and J. K. Loosli. McGraw-Hill Book Co., New York, 1956.

Appendix

TABLE A–1. Recommended Daily Dietary Allowances for Human Beings* [1]

(For the Maintenance of Normally Active, Healthy Persons in the U.S.A.)

	Age, Years	Weight, kg. (lb.)	Height, cm. (in.)	Calories	Protein, g.	Calcium, g.	Iron, mg.	Vitamin A, I.U.	Thiam., mg.	Ribo., mg.	Niacin,[2] mg. equiv.	Asc. Acid, mg.	Vitamin D, I.U.
Men	25	70 (154)	175 (69)	3,200[3]	70	0.8	10	5,000	1.6	1.8	21	75	
	45	70 (154)	175 (69)	3,000	70	0.8	10	5,000	1.5	1.8	20	75	
	65	70 (154)	175 (69)	2,550	70	0.8	10	5,000	1.3	1.8	18	75	
Women	25	58 (128)	163 (64)	2,300	58	0.8	12	5,000	1.2	1.5	17	70	
	45	58 (128)	163 (64)	2,200	58	0.8	12	5,000	1.1	1.5	17	70	
	65	58 (128)	163 (64)	1,800	58	0.8	12	5,000	1.0	1.5	17	70	
	Pregnant (second half)			+300	+20	1.5	15	6,000	1.3	2.0	+3	100	400
	Lactating (850 ml. daily)			+1,000	+40	2.0	15	8,000	1.7	2.5	+2	150	400
Infants[4]	0–1/12[4]				See Footnote 4								
	2/12–6/12	6 (13)	60 (24)	kg. × 120		0.6	5	1,500	0.4	0.5	6	30	400
	7/12–12/12	9 (20)	70 (28)	kg. × 100		0.8	7	1,500	0.5	0.8	7	30	400
Children	1–3	12 (27)	87 (34)	1,300	40	1.0	7	2,000	0.7	1.0	8	35	400
	4–6	18 (40)	109 (43)	1,700	50	1.0	8	2,500	0.9	1.3	11	50	400
	7–9	27 (60)	129 (51)	2,100	60	1.0	10	3,500	1.1	1.5	14	60	400
	10–12	36 (79)	144 (57)	2,500	70	1.2	12	4,500	1.3	1.8	17	75	400
Boys	13–15	49 (108)	163 (64)	3,100	85	1.4	15	5,000	1.6	2.1	21	90	400
	16–19	63 (139)	175 (69)	3,600	100	1.4	15	5,000	1.8	2.5	25	100	400
Girls	13–15	49 (108)	160 (63)	2,600	80	1.3	15	5,000	1.3	2.0	17	80	400
	16–19	54 (120)	162 (64)	2,400	75	1.3	15	5,000	1.2	1.9	16	80	400

* Food and Nutrition Board, National Academy of Sciences, National Research Council, revised 1958.

[1] The allowance levels are intended to cover individual variations among most normal persons as they live in the United States under usual environmental stresses. The recommended allowances can be attained with a variety of common foods, providing other nutrients for which human requirements have been less well defined.

[2] Niacin equivalents include dietary sources of the preformed vitamin and the precursor, tryptophan. 60 mg. tryptophan equals 1 mg. niacin.

[3] Calorie allowances apply to individuals usually engaged in moderate physical activity. For office workers or others in sedentary occupations they are excessive. Adjustments must be made for variations in body size, age, physical activity, and environmental temperature.

[4] The Board recognizes that human milk is the natural food for infants and feels that breast feeding is the best and desired procedure for meeting nutrient requirements in the first months of life. No allowances are stated for the first month of life. Breast feeding is particularly indicated during the first month when infants show handicaps in homeostasis due to different rates of maturation of digestive, excretory, and endocrine functions. Recommendations as listed pertain to nutrient intake as afforded by cow's milk formulas and supplementary foods given the infant when breast feeding is terminated. Allowances are not given for protein during infancy.

TABLE A–2. Chemical Composition of Selected Human Foods

(Nutritive Value of 100 g., Edible Portion)

Food Item	Water, %	Food Energy, Cal.	Protein, g.	Fat, g.	Carbohydrate, g.	Calcium, mg.	Phosphorus, mg.	Iron, mg.	Vitamin A Value, I.U.	Thiamine, mg.	Riboflavin, mg.	Niacin, mg.	Ascorbic Acid, mg.
Milk, cream, ice cream, cheese													
Milk													
Dry whole	3.5	496	25.8	26.7	38.0	949	728	0.58	1,400	0.30	1.46	0.7	6
Evaporated, unsweetened	73.7	139	7.0	7.9	9.9	243	195	0.17	400	0.05	0.36	0.2	1
Fresh skim	90.5	35	3.5	0.1	5.1	118	93	0.07	Trace	0.04	0.18	0.1	1
Fresh whole	87.0	69	3.5	3.9	4.9	118	93	0.07	160	0.04	0.17	0.1	1
Cream, ice cream													
Cream (20 per cent), sweet or sour	72.5	208	2.9	20.0	4.0	97	77	0.06	830	0.03	0.14	0.1	1
Ice cream, plain	62.0	210	4.0	12.3	20.8	132	104	0.10	540	0.04	0.19	0.1	Trace
Cheese													
Cheddar type	39	393	23.9	32.3	1.7	873	610	0.57	1,740	0.04	0.50	0.2	0
Cottage	74.0	101	19.2	0.8	4.3	82	263	0.46	30	0.02	0.29	0.1	0
Fats, oils													
Bacon, medium fat	10	626	9.1	65	1.1	13	108	0.8	0	0.42	0.10	2.1	0
Butter	15.5	733	0.6	81	0.4	16	16	0.2	3,300	Trace	0.01	0.1	0
Lard, other shortening	0	900	0	100	0	0	0	0	0	0	0	0	0
Margarine with vitamin A added	15.5	733	0.6	81	0.4	2	15	0.2	1,980	0	0	0	0
Salt pork, fat	8	781	3.9	85	0	2	42	0.6	0	0.18	0.04	0.9	0
Eggs													
Whole, dried	2	593	48.2	43.3	2.6	187	800	8.7	4,450	0.35	1.23	0.2	0
Whole, fresh	74.0	158	12.8	11.5	0.7	54	210	2.7	1,140	0.12	0.34	0.1	0
Meat, poultry, fish													
Beef													
Loin steaks (wholesale loin)	57	293	16.9	25	0	10	182	2.5	0	0.10	0.13	4.6	0
Round steak (wholesale round)	67	194	19.3	13	0	11	208	2.9	0	0.12	0.15	5.2	0
Lamb													
Leg roast (wholesale leg)	63.7	230	18.0	17.5	0	10	194	2.7	0	0.21	0.26	5.9	0
Sirloin chop (wholesale leg)	63.7	230	18.0	17.5	0	10	194	2.7	0	0.21	0.26	5.9	0
Pork													
Ham, fresh	53	340	15.2	31	0	9	164	2.3	0	0.96	0.19	4.1	0
Ham, smoked	42	384	16.9	35	0.3	10	182	2.5	0	0.78	0.19	3.8	0
Pork links, sausage	41.9	446	10.8	44.8	0	6	116	1.6	0	0.22	0.15	2.3	0
Poultry													
Chicken, roasters	66.0	194	20.2	12.6	0	16	218	1.9	Trace	0.11	0.18	8.6	
Turkey, medium fat	58.3	262	20.1	20.2	0	23	320	3.8	Trace	0.12	0.19	7.9	
Fish and shellfish													
Cod	82.6	70	16.5	0.4	0	18	189	0.9	0	0.04	0.05	2.3	2
Salmon, canned	67.4	169	20.6	9.6	0	67	286	1.3	80	0.03	0.18	6.5	0

Dry beans and peas, nuts													
Dry beans and peas													
Beans, canned, baked	71.0	117	5.7	2.0	19.0	40	154	3.4	70	0.05	0.05	0.8	4
Beans, lima, dry seed	12.6	341	20.7	1.3	61.6	68	381	7.5	0	0.60	0.24	2.1	2
Peas, split	10.0	354	24.5	1.0	61.7	73	397	6.0	370	0.87	0.29	3.9	2
Nuts													
Peanut butter	1.7	619	26.1	47.8	21.0	74	393	1.9	0	0.20	0.16	16.2	0
Peanuts, roasted	2.6	600	26.9	44.2	23.6	74	393	1.9	0	0.30	0.16	16.2	0
Fresh vegetables													
Asparagus	93.0	26	2.2	0.2	3.9	21	62	0.9	1,000	0.16	0.17	1.2	33
Beans, snap	88.9	42	2.4	0.2	7.7	65	44	1.1	630	0.08	0.10	0.6	19
Beets	87.6	46	1.6	0.1	9.6	27	43	1.0	20	0.03	0.05	0.4	10
Carrots	88.2	45	1.2	0.3	9.3	39	37	0.8	12,000	0.07	0.06	0.5	6
Corn, sweet, white, or yellow	73.9	108	3.7	1.2	20.5	9	120	0.5	390	0.15	0.14	1.4	12
Cucumbers	96.1	14	.7	0.1	2.7	10	21	0.3	0	0.04	0.09	0.2	8
Lettuce, headed	94.8	18	1.2	0.2	2.9	22	25	0.5	540	0.06	0.07	0.2	8
Onions, mature	87.5	49	1.4	0.2	10.3	32	44	0.5	50	0.03	0.02	0.1	9
Peas, green	74.3	101	6.7	0.4	17.7	22	122	1.9	680	0.36	0.18	2.1	26
Potatoes	77.8	85	2.0	0.1	19.1	11	56	0.7	20	0.11	0.04	1.2	17
Spinach	92.7	25	2.3	0.3	3.2	81	55	3.0	9,420	0.12	0.24	0.7	59
Sweet potatoes	68.5	125	1.8	0.7	27.9	30	49	0.7	7,700	0.10	0.06	0.7	22
Tomatoes	94.1	23	1.0	0.3	4.0	11	27	0.6	1,100	0.06	0.04	0.6	23
Turnips	90.9	35	1.1	0.2	7.1	40	34	0.5	Trace	0.06	0.06	0.5	28
Fresh fruit													
Apples	84.1	64	0.3	0.4	14.9	6	10	0.3	90	0.04	0.02	0.2	5
Bananas	74.8	99	1.2	0.2	23	8	28	0.6	430	0.09	0.06	0.6	10
Strawberries	90.0	41	0.8	0.6	8.1	28	27	0.8	60	0.03	0.07	0.3	60
Grapefruit	88.8	44	0.5	0.2	10.1	17	18	0.3	Trace	0.04	0.02	0.2	40
Lemons	89.3	44	0.9	0.6	8.7	14	10	0.1	0	0.04	Trace	0.1	45
Oranges	87.2	50	0.9	0.2	11.2	33	23	0.4	190	0.08	0.03	0.2	42
Peaches	86.9	51	0.5	0.1	12.0	8	22	0.6	880	0.02	0.05	0.9	8
Rhubarb	94.9	18	0.5	0.1	3.8	51	25	0.5	30	0.01		0.1	9
Grain products													
Flour													
Wheat, patent	12	355	10.8	0.9	75.9	19	93	0.7	0	0.07	0.03	0.8	0
Wheat, patent, enriched	12	355	10.8	0.9	75.9	19	93	2.9	0	0.44	0.26	3.5	0
Whole wheat	11	360	13.0	2.0	72.4	38	385	3.8	0	0.56	0.12	5.6	0
Breakfast cereals													
Corn flakes	9.3	359	7.9	0.7	80.3	10	56	1.0	0	0.16	0.08	1.6	0
Oatmeal	8.3	396	14.2	7.4	68.2	54	365	5.2	0	0.55	0.14	1.1	0
Shredded wheat	7.7	369	10.4	1.4	78.7	38	385	3.8	0	0.20	0.14	4.2	0
Other cereals													
Hominy	11.4	357	8.5	0.8	78.9	11	70	1.0	0	0.15	0.05	0.9	0
Macaroni, spaghetti	11	360	13	1.4	73.9	22	144	1.2	0	0.13	0.08	2.1	0
Sugars, sweets													
Honey	20	319	0.3	0	79.5	5	16	0.9	0	Trace	0.04	0.2	4
Sugar, granulated or powdered	0.5	398	0	0	99.5	0	0	0.1	0	0	0	0	0
Miscellaneous													
Cocoa	4.3	329	9.0	18.8	31.0	. . .	709	2.7	0	Trace	0.39	2.3	0
Yeast, dried, brewers'	7.0	348	46.1	1.6	37.4	106	1,893	18.2	0	9.69	5.45	36.2	0

TABLE A–3. Daily Nutrient Requirements of Dairy Cattle*

(Based on Air-dry Feed Containing 90 Per Cent Dry Matter)

	Daily Gain		Daily Nutrients per Animal[1]								
Body Weight, lb.	Small Breeds, lb.	Large Breeds, lb.	Feed, lb.	Protein, lb.	Digestible Protein, lb.	TDN,[2] lb.	DE,[2] therm.	Ca, g.	P, g.	Carotene, mg.	Vitamin D, I.U.
Normal Growth of Dairy Heifers											
50	0.5	...	0.9	0.31	0.20	1.0	2.0	4	3	2[3]	150
100	1.0	0.8	2.0	0.62	0.40	2.0	4.0	7	6	4	300
150	1.3	1.4	4.0	0.78	0.50	3.0	6.1	12	10	6	450
200	1.4	1.6	6.0	0.94	0.60	4.0	8.1	13	10	8	600
400	1.2	1.8	11.0	1.25	0.80	6.5	13.1	13	12	16	(4)
600	0.8	1.4	15.0	1.33	0.85	8.5	17.1	13	12	24	...
800	1.1	1.2	19.0	1.40	0.90	10.0	20.2	13	12	32	...
1,000	...	1.3	22.0	1.48	0.95	11.0	22.2	12	12	40	...
1,200	...	1.2	24.0	1.56	1.00	12.0	24.2	12	12	48	...
Maintenance of Mature Cows[5]											
800	...	...	12	0.95	0.50	5.8	11.7	6	6	32	(4)
1,000	...	...	14	1.13	0.60	7.0	14.1	8	8	40	...
1,200	...	...	16	1.32	0.70	8.2	16.6	10	10	48	...
1,400	...	...	19	1.51	0.80	9.4	19.0	11	11	56	...
1,600	...	...	21	1.64	0.87	10.5	21.2	12	12	64	...
Reproduction (Add to Maintenance during Last 2 to 3 Months)											
...	2.0	2.0	8.0	1.13	0.60	6.0	12.1	8	7	30	(4)
Lactation (Add to Maintenance for Each Pound of Milk)											
...	...	3.0% fat	...	0.062	0.040	0.28	0.57	1	0.7	(6)	(6)
...	...	4.0% fat	...	0.070	0.045	0.32	0.65	1	0.7	...	...
...	...	5.0% fat	...	0.078	0.050	0.37	0.75	1	0.7	...	...
	...	6.0% fat	...	0.086	0.055	0.42	0.85	1	0.7	...	...
Maintenance of Breeding Bulls											
1,200	...	...	18	1.56	1.00	10.3	20.3	10	10	48	...
1,600	...	...	22	1.87	1.20	12.9	26.1	12	12	64	...
2,000	...	...	27	2.20	1.45	15.6	31.5	16	16	80	...
2,400	...	...	31	2.50	1.60	18.2	36.8	19	19	96	...

* Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1956.

[1] Thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, folic acid, vitamin B_{12}, and vitamin K are synthesized by bacteria in the rumen, and it appears that adequate amounts of these vitamins are furnished by a combination of rumen synthesis and natural feedstuffs. Manganese, magnesium, iron, copper, and cobalt are essential, and the amounts needed are discussed in the text.

[2] DE (digestible energy) was calculated on the assumption that 1 g. of TDN (total digestible nutrients) has 4.45 kcal. of digestible energy. DE may be converted to metabolizable energy by multiplying by 82 per cent. The therm equals 1,000 large cal.

[3] Calves should receive colostrum the first few days after birth, as a source of vitamin A and other essential factors.

[4] While vitamin D is known to be required, the data are inadequate to warrant specific figures for older growing animals and for maintenance, reproduction, and lactation.

[5] When calculating the intakes for lactating heifers that are still growing, it is recommended that the figure for growth rather than maintenance be used.

[6] When adequate amounts of vitamins A and D are fed for normal reproduction, extra amounts will not timulate milk production but will increase the vitamin content of the milk.

TABLE A–4. Daily Nutrient Requirements of Beef Cattle*

(Based upon Air-dry Feed Containing 90 Per Cent Dry Matter)

			Daily Nutrients per Animal							
Body Weight, lb.	Av. Daily Gain,[1] lb.	Daily Feed per Animal, lb.	Total Protein, lb.	Digestible Protein, lb.	TDN,[2] lb.	DE,[2] therm.	Ca, g.	P, g.	Carotene, mg.	Vitamin A,[3] IU × 1,000
Fattening Calves Finished as Short Yearlings										
400	2.3	12	1.3	1.0	8.0	16	20	15	7	2.8
600	2.4	16	1.8	1.3	10.9	22	20	17	10	4.0
800	2.2	20	2.0	1.5	13.6	27	20	18	14	5.6
1,000	2.2	22	2.2	1.6	15.0	30	20	20	17	6.8
Fattening Yearling Cattle										
600	2.4	18	1.8	1.4	11.7	23	20	17	10	4.0
800	2.8	22	2.2	1.6	14.3	29	20	20	14	5.6
1,000	2.5	26	2.6	2.0	16.9	34	20	24	17	6.8
1,100	2.3	27	2.7	2.0	17.6	35	20	25	19	7.6
Fattening Two-Year Cattle										
800	2.8	24	2.4	1.8	14.9	30	20	22	14	5.6
1,000	3.0	27	2.7	2.0	16.7	33	20	25	17	6.8
1,200	2.6	29	2.9	2.2	18.0	36	20	26	20	8.0
Wintering Weanling Calves										
400	1.0	11	1.1	0.7	6.0	12	13	10	7	2.8
500	1.0	13	1.3	0.8	7.0	14	13	10	9	3.6
600	1.0	15	1.4	0.8	8.0	16	13	10	10	4.0
Wintering Yearling Cattle										
600	1.0	16	1.3	0.8	8.0	16	13	11	10	4.0
800	0.7	18	1.4	0.8	9.0	18	13	12	14	5.6
900	0.5	18	1.4	0.8	9.0	18	13	12	15	6.0
Wintering Pregnant Heifers										
700	1.5	20	1.5	0.9	10.0	20	15	14	28	11.2
900	0.8	18	1.4	0.8	9.0	18	13	12	36	14.4
1,000	0.5	18	1.4	0.8	9.0	18	13	12	40	16.0
Wintering Mature Pregnant Cows[4]										
800	1.5	22	1.6	1.0	11.0	22	16	15	32	12.8
1,000	0.4	18	1.4	0.8	9.0	18	13	12	40	16.0
1,200	0.0	18	1.4	0.8	9.0	18	13	12	48	19.2

TABLE A–4. (Continued)

Body Weight, lb.	Av. Daily Gain,[1] lb.	Daily Feed per Animal, lb.	Daily Nutrients per Animal							
			Total Protein, lb.	Digestible Protein, lb.	TDN,[2] lb.	DE,[2] therm.	Ca, g.	P, g.	Carotene, mg.	Vitamin A,[3] IU × 1,000
Cows Nursing Calves, First 3–4 Months Postpartum										
900–1,100	0.0	28	2.3	1.4	16.8	34	30	23	100	40.0
Normal Growth Heifers and Steers										
400	1.6	12	1.4	0.8	7.0	14	16	11	7	2.8
600	1.4	16	1.5	0.9	8.5	17	15	12	10	4.0
800	1.2	19	1.5	0.9	9.5	19	15	13	14	5.6
1,000	1.0	21	1.6	1.0	10.5	21	13	14	17	6.8
Bulls, Growth and Maintenance (Moderate Activity)										
600	2.3	16	2.0	1.2	10.1	20	21	15	36	14.4
1,000	1.6	20	2.4	1.4	12.0	24	19	15	60	24.0
1,400	1.0	24	2.4	1.4	14.2	28	17	16	84	33.6
1,800	0.0	26	2.4	1.5	14.0	28	15	18	108	43.2

* Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1958.

[1] Average daily gain for fattening cattle is based upon cattle receiving stilbestrol. Fattening cattle not receiving stilbestrol gain from 10 to 20 per cent slower than the indicated values.

[2] DE (digestible energy) was calculated on the assumption that 1 g. of TDN (total digestible nutrients) has 4.41 kcal. (large calorie) of digestible energy. New information suggested that the value of 4.41 is more accurate than the 4.45 value often used previously. DE may be converted to metabolizable energy by multiplying by 82 per cent. 1,000 large cal. equal 1 therm.

[3] Multiply figures in column by 1,000. Vitamin A requirement computed on the basis of 1 mg. carotene equals 400 I.U. Vitamin A.

[4] Under some range conditions it may not be economically justifiable to feed pregnant cows during winter months at these levels of total feed and TDN. Short periods of moderate weight loss can be tolerated without serious effects.

TABLE A–5. Daily Nutrient Requirements of Sheep*

(Based on Air-dry Feed Containing 90 Per Cent Dry Matter)

Body Weight, lb.	Gain or Loss, lb.	Feed		Daily Nutrients per Animal									
		Per Animal, lb.	% Live Weight	Protein, lb.	DP,[1] lb.	TDN,[2] lb.	DE,[2] therm.	Ca, g.	P, g.	Salt, g.	Carotene, mg.	Vit. A, I.U.	Vit. D, I.U.
Ewes—Non-lactating and First 15 Weeks of Gestation													
100	0.07	2.6	2.6	0.20	0.11	1.3	2.6	3.2	2.5	9.0	1.7	965	250
120	0.07	3.0	2.5	0.23	0.13	1.5	3.0	3.3	2.6	10.0	2.0	1,156	300
140	0.07	3.4	2.4	0.27	0.15	1.7	3.4	3.4	2.7	11.0	2.4	1,350	350
160	0.07	3.8	2.4	0.29	0.16	1.9	3.8	3.5	2.8	12.0	2.7	1,542	400
Ewes—Last 6 Weeks of Gestation													
100	0.37	3.8	3.8	0.31	0.17	2.0	4.0	4.2	3.1	10.0	5.8	2,316	250
120	0.37	4.2	3.5	0.32	0.18	2.2	4.4	4.4	3.3	11.0	6.8	2,775	300
140	0.37	4.6	3.3	0.36	0.20	2.4	4.8	4.6	3.5	12.0	7.9	3,240	350
160	0.37	4.8	3.0	0.36	0.20	2.5	5.0	4.8	3.7	13.0	9.1	3,702	400
Ewes—First 8–10 Weeks of Lactation													
100	−0.08	4.6	4.6	0.40	0.22	2.7	5.5	6.2	4.6	11.0	5.8	2,316	250
120	−0.08	5.0	4.2	0.42	0.23	2.9	5.9	6.5	4.8	12.0	6.8	2,775	300
140	−0.08	5.4	4.0	0.45	0.25	3.1	6.3	6.8	5.0	13.0	7.9	3,240	350
160	−0.08	5.6	3.5	0.47	0.26	3.2	6.5	7.1	5.2	14.0	9.1	3,702	400
Ewes—Last 12–14 Weeks of Lactation													
100	0.07	3.8	3.8	0.31	0.17	2.0	4.0	4.6	3.4	10.0	5.8	2,316	250
120	0.07	4.2	3.5	0.32	0.18	2.2	4.4	4.8	3.6	11.0	6.8	2,775	300
140	0.07	4.6	3.3	0.34	0.19	2.4	4.8	5.0	3.8	12.0	7.9	3,240	350
160	0.07	4.8	3.0	0.36	0.20	2.5	5.0	5.2	4.0	13.0	9.1	3,702	400
Ewes—Replacement Lambs													
60	0.30	2.7	4.5	0.29	0.16	1.6	3.2	2.9	2.6	8.0	1.7	696	150
80	0.20	3.2	4.0	0.27	0.15	1.7	3.4	3.0	2.7	9.0	2.3	926	200
100	0.14	3.4	3.4	0.25	0.14	1.7	3.4	3.1	2.8	10.0	2.8	1,158	250
120	0.07	3.4	2.8	0.25	0.14	1.7	3.4	3.2	2.9	11.0	3.4	1,388	300
Rams—Lambs and Yearlings													
80	0.40	3.2	4.0	0.31	0.17	2.0	4.0	3.0	2.7	9.0	2.3	926	200
100	0.30	3.7	3.7	0.31	0.17	2.1	4.2	3.1	2.8	10.0	2.8	1,158	250
120	0.20	4.2	3.5	0.31	0.17	2.2	4.4	3.2	2.9	11.0	3.4	1,388	300
140	0.10	4.6	3.3	0.32	0.18	2.3	4.6	3.3	3.0	11.0	4.0	1,620	350
160	0.10	4.8	3.0	0.32	0.18	2.4	4.8	3.4	3.1	12.0	4.5	1,851	400
Lambs—Fattening													
60	0.30	2.7	4.5	0.31	0.17	1.6	3.2	2.9	2.6	8.0	1.0	580	150
70	0.40	3.1	4.4	0.33	0.18	1.9	3.8	2.9	2.6	8.0	1.2	674	175
80	0.40	3.4	4.3	0.35	0.19	2.1	4.2	3.0	2.7	9.0	1.4	771	200
90	0.40	3.8	4.2	0.36	0.20	2.4	4.8	3.0	2.7	9.0	1.5	868	225
100	0.35	4.0	4.0	0.36	0.20	2.6	5.2	3.1	2.8	10.0	1.7	965	250

* Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1957.

[1] DP = digestible protein.

[2] DE (digestible energy) was calculated on the assumption that 1 g. of TDN (total digestible nutrients) has 4.45 kcal. of digestible energy. D.E. may be converted to metabolizable energy by multiplying by 82 per cent. The therm equals 1,000 large cal.

TABLE A–6. Chemical Composition of Selected Ruminant Feeds*

Feed	Dry Matter, %	Crude Protein, %	Total Digestible Nutrients, %	Digestible Protein, %	Calcium, %	Phosphorus, %	Carotene, mg./lb.
Air-dry forages							
Alfalfa hay	92.8	15.4	50.4	10.8	1.51	0.21	8.2
Alfalfa meal, dehydrated	93.4	16.9	53.0	12.5	1.56	0.22	44.6
Clover hay, red	87.1	13.6	49.6	9.7	1.01	0.24	11.0
Corn stover, very dry	89.3	5.8	48.6	1.9	0.45	0.10	...
Lespedeza hay	88.0	12.3	45.5	5.4	0.97	0.23	22.4
Oat straw	91.9	4.4	45.5	1.5	0.23	0.20	0.1
Prairie hay, moderately green	90.4	5.7	49.2	2.6	0.50	0.10	11.5
Soybean hay	90.8	14.8	50.6	11.1	1.26	0.22	13.6
Timothy hay	88.7	6.2	46.9	2.9	0.31	0.13	9.2
Silages, roots, tubers							
Alfalfa silage, slightly wilted	31.1	5.7	18.8	4.0	0.38	0.06	14.9
Corn silage, well matured	29.1	2.4	19.0	1.0	0.08	0.08	4.0
Potatoes	21.1	2.1	17.0	0.7	0.01	0.06	...
Grains, seeds, and by-product concentrates							
Barley	90.4	12.8	77.1	10.0	0.07	0.32	...
Brewers' grains, dried (18–23% protein)	92.1	20.7	68.2	15.5	0.16	0.47	...
Citrus pulp	90.5	6.5	70.5	2.0	2.08	0.11	...
Corn yellow, No. 2 equivalent	85.2	9.4	80.6	7.1	0.01	0.25	2.2
Corn gluten meal	92.0	43.0	78.4	37.8	0.10	0.47	7.4
Cottonseed meal (38–43% protein)	92.7	41.0	73.6	33.0	0.19	1.11	...
Distillers' corn grains, dried	93.2	28.1	81.6	20.1	0.04	0.29	1.4
Linseed meal (33–38% protein)	91.5	35.3	76.0	29.7	0.36	0.84	...
Molasses, cane	74.1	2.8	56.6	0.9	0.56	0.06	...
Oats	92.3	12.5	72.3	9.1	0.10	0.40	...
Peanut oil meal (38–43% protein)	93.6	41.6	78.8	37.0	0.10	0.50	...
Soybean oil meal (hyd. or exp.)	92.2	41.7	77.4	37.5	0.29	0.67	...
Wheat	89.4	12.0	78.6	9.1	0.05	0.38	...
Wheat bran	90.6	16.4	63.3	13.1	0.10	0.14	1.2

TABLE A–7. Daily Nutrient Requirements for Swine*

	Description of Pigs									
	Market Stock						Breeding Stock			
							Pregnant Females and Breeding Boars		Lactating Females	
							Young Stock	Adults	Gilts	Adults
Liveweight, lb.	25	50	100	150	200	250	300	500	350	450
Expected daily gain, lb.	0.8	1.2	1.6	1.8	1.8	1.8	0.75	0.5	...	...
Total feed (air dry), lb.	2.0	3.2	5.3	6.8	7.5	8.3	6.0	7.5	11.0	12.5
Total digestible nutrients (75% TDN[1]), lb.	1.6[1]	2.4	4.0	5.1	5.6	6.2	4.5	5.6	8.3	9.4
Crude protein, lb.	0.36	0.51	0.74	0.88	0.90	1.00	0.90	1.05	1.65	1.75
Inorganic nutrients										
Calcium, g.	7.3	9.4	15.6	17.0	18.7	20.7	16.3	20.4	30.0	34.0
Phosphorus, g.	5.4	6.5	10.8	10.2	11.2	12.4	10.9	13.6	20.0	22.7
Salt (NaCl), g.	4.5	7.3	12.0	15.4	17.0	18.8	13.6	17.0	25.0	28.4
Vitamins										
Carotene, mg.	0.5	1.0	2.0	3.0	4.0	5.0	15.0	18.7	27.5	31.2
Vitamin D, I.U.	180.0	288.0	477.0	612.0	675.0	747.0	540.0	675.0	990.0	1,125.0
Thiamine, mg.	1.0	1.6	2.6	3.4	3.8	4.2	3.0	3.8	5.5	6.2
Riboflavin, mg.	2.4	3.2	5.3	6.8	7.5	8.3	7.2	9.0	13.2	15.0
Niacin, mg.	16.0	19.2	26.5	34.0	37.5	41.5	30.0	37.5	55.0	62.5
Pantothenic acid, mg.	10.0	16.0	23.8	30.6	33.8	37.4	27.0	33.8	49.5	56.2
Pyridoxine, mg.	1.2	1.9	...	...	...	...	...	...	...	...
Choline, mg.	800.0	...	...	...	...	...	...	...	...	...
Vitamin B_{12}, μg.	20.0	16.0	26.5	...	...	...	...	...	...	

* Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1953.

[1] TDN = total digestible nutrients. For young pigs a high-energy diet (80% TDN) is recommended.

TABLE A–8. Partial Composition of Some Hog Feeds*

(Air-dry Basis)

Feed	Energy and Protein		Inorganic Nutrients			Vitamins per Pound Feedstuff				
	TDN,[1] %	Crude Protein, %	Calcium, %	Phosphorus, %	Carotene, mg.	Thiamine, mg.	Riboflavin, mg.	Niacin, mg.	Pantothenic Acid, mg.	Choline, mg.
Grains and other carbohydrates										
Barley, excl. P. C.	72	12.7	0.09	0.47	...	1.7	0.8	24.1	3.7	530
Barley, P.C.	70	9.7	0.06	0.41	...	1.8	0.6	20.0	3.3	425
Corn, No. 2, yellow	78	8.6	0.02	0.27	2.0	1.8	0.5	10.2	2.7	220
Oats	65	12.0	0.09	0.43	...	2.9	0.6	8.2	6.8	480
Rye	75	12.6	0.01	0.33	...	2.0	0.7	7.1	4.2	...
Sorghum, grain	78	11.3	0.03	0.30	...	1.7	0.6	21.3	5.0	...
Wheat	80	13.5	0.05	0.37	...	2.2	0.5	26.6	6.0	400
Potato meal, sweet	88	7.4	...	...	...	...	...	...	...	160
Potato meal, white	82	8.2	0.08	0.22	...	...	...	...	...	480
Mill concentrates										
Hominy feed, yellow	80	11.2	0.05	0.66	4.1	3.8	1.0	22.6	3.5	...
Rice bran	71	12.8	0.08	1.36	...	10.3	1.4	163.0	17.9	...
Rice polish	88	12.4	0.03	1.10	...	9.1	1.0	235.0	5.5	572
Rye middlings	74	16.7	...	...	...	1.5	1.0	7.8	10.5	...
Molasses, cane	58	3.1	0.74	0.08	...	0.4	1.0	21.3	17.9	390
Wheat standard middlings	65	17.6	0.09	0.90	1.4	7.5	1.1	56.1	7.1	600
Wheat red dog	73	18.3	0.06	0.47	...	9.7	1.6	25.1	6.2	...
Wheat bran	57	16.4	0.14	1.30	...	3.6	1.2	126.5	13.2	648
Protein supplements (plant)										
Cottonseed meal (41%)	72	41.0	0.23	1.18	0.1	5.0	2.5	13.0	6.4	1,525
Linseed meal (35%)	68	35.4	0.38	0.86	0.1	3.5	1.5	16.8	8.0	650
Peanut meal (41%)	82	41.6	0.10	0.50	0.1	2.6	1.6	96.6	24.7	1,025
Soybean meal (exp. hyd. 41%)	79	42.0	0.24	0.63	0.1	6.1	1.7	11.4	10.1	1,330
Soybean meal (solv. 43%)	78	46.0	0.25	0.68	0.1	6.1	1.7	11.4	10.1	1,330
Distillers' solubles, dried	77	28.0	0.35	1.40	0.3	3.2	6.1	58.0	10.6	...
Yeast, brewers', dried	71	46.8	0.11	1.52	...	43.0	13.0	213.6	49.1	1,620

Protein supplements (animal)										
Buttermilk, dried	77	32.4	1.35	0.94	...	1.7	13.7	2.8	13.5	1,050
Fish meal, menhaden	65	62.2	5.00	3.40	...	0.2	2.4	25.9	...	...
Fish meal, sardine	68	67.2	4.21	2.54	...	0.2	2.5	26.0	1.3	1,280
Meat and bone scrap (50%)	67	30.6	9.70	4.20	...	...	2.1	24.8	2.4	750
Meat scrap (60%)	72	60.9	6.30	3.50	...	0.1	2.5	23.7	2.8	...
Skimmed milk, dried	86	34.7	1.27	0.10	...	1.5	10.0	5.7	16.0	745
Tankage (60%)	71	60.6	6.11	3.01	...	0.1	1.2	19.2	1.2	1,047
Whey, dried	83	12.2	0.91	0.75	...	1.8	13.0	5.1	22.4	1,000
Miscellaneous										
Alfalfa meal, sun cured (17%)	30	17.6	1.50	0.30	20.0	1.1	5.0	16.1	12.7	...
Alfalfa leaf meal, sun cured (20%)	34	20.9	1.42	0.25	29.0	1.8	7.0	24.0	...	600
Alfalfa meal, dehydrated (17%)	33	17.8	1.70	0.28	36.0	1.5	7.3	18.3	17.4	590
Bone meal, steamed	...	6.2	29.30	13.60	...	0.9	0.4	2.0	0.8	
Bone meal, special steamed	...	11.1	28.50	13.50	...	1.0	0.4	2.0	0.8	

* The data on the average composition of feedstuffs are taken from the figures supplied by the Committee on Feed Composition of the National Research Council, Schneider's Feeds of the World, and compilations of data in the laboratories of the Bureau of Animal Industry, U. S. D. A. See Nutrient Requirements for Swine, *Publication* 295, Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1953.

[1] TDN = total digestible nutrients.

TABLE A–9. Nutrient Requirements for Chickens[*] [1]

(In Percentage or Amount Per Pound of Feed)

Nutrient	Starting Chickens 0–8 Weeks	Growing Chickens 8–18 Weeks	Laying Hens	Breeding Hens
Total protein, %	20	16	15	15
Vitamins				
Vitamin A activity (U.S.P. units)[2]	1,200	1,200	2,000	2,000
Vitamin D (I.C.U.)	90	90	225	225
Thiamine, mg.	0.8	?	?	?
Riboflavin, mg.	1.3	0.8	1.0	1.7
Pantothenic acid, mg.	4.2	4.2	2.1	4.2
Niacin, mg.	12	?	?	?
Pyridoxine, mg.	1.3	?	1.3	1.3
Biotin, mg.	0.04	?	?	?
Choline, mg.	600	?	?	?
Folacin, mg.	0.25	?	0.11	0.16
Minerals				
Calcium, %	1.0	1.0	2.25[3]	2.25[3]
Phosphorus, %	0.6	0.6	0.6	0.6
Salt, %	0.5	0.5	0.5	0.5
Potassium, %	0.2	0.16	?	?
Manganese, mg.	25	?	?	15
Iodine, mg.	0.5	0.2	0.2	0.5
Magnesium, mg.	220	?	?	?

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[*] Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1954.

[1] These figures are estimates of requirements and include no margins of safety.

[2] May be vitamin A or pro-vitamin A.

[3] This amount of calcium need not be incorporated in the mixed feed, inasmuch as calcium supplements fed free choice are considered as part of the ration.

[4] At least 0.45% of the total feed of starting chickens should be inorganic phosphorus. All the phosphorus of non-plant feed ingredients is considered to be inorganic. Approximately 30% of the phosphorus of plant products is non-phytin phosphorus and may be considered as part of the inorganic phosphorus required. A portion of the phosphorus requirement of growing chickens and laying and breeding hens must also be supplied in inorganic form. For birds in these categories the requirement for inorganic phosphorus is lower and not as well defined as for starting chickens.

[5] This figure represents salt or sodium chloride added as such or in marine or fermentation products of high sodium chloride content.

TABLE A–10. Nutrient Requirements for Turkeys* [1]

(In Percentage or Amount per Pound of Feed)

Nutrient	Starting Poults 0–8 Weeks	Growing Turkeys 8–16 Weeks	Breeding Turkeys
Total protein, %[2]	28	20	15
Vitamins			
Vitamin A activity (U.S.P.)[3]	2,400	2,400	2,400
Vitamin D (I.C.U.)	400	400	400
Riboflavin, mg.	1.7	?	1.5
Pantothenic acid, mg.	5.0	?	?
Choline, mg.	750	?	?
Folacin, mg.	0.4	?	?
Minerals			
Calcium, %	2.0	2.0	2.25[4]
Phosphorus, %[5]	1.0	1.0	0.75
Manganese, mg.	25	?	15
Salt, %[6]	0.5	0.5	0.5

* Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1954.

[1] These figures are estimates of requirements and include no margins of safety.

[2] The protein content of rations for growing turkeys from 16 weeks to market weight may be reduced to 16%.

[3] May be vitamin A or pro-vitamin A.

[4] This amount of calcium need not be incorporated in the mixed feed inasmuch as calcium supplements fed free choice are considered as part of the ration.

[5] At least 0.50% of the total feed of starting poults should be inorganic phosphorus. All the phosphorus of non-plant feed ingredients is considered to be inorganic. Approximately 30% of the phosphorus of plant products is non-phytin phosphorus and may be considered as part of the inorganic phosphorus required. Presumably a portion of the requirement of growing and breeding turkeys must also be furnished in inorganic form.

[6] This figure represents salt or sodium chloride added as such or in marine or fermentation products of high sodium chloride content.

TABLE A–11. Nutrient Requirements for Ducks* [1]

(In Percentage or Amount per Pound of Feed)

Nutrient	Starting and Growing Ducks
Total protein, %	17
Vitamins	
Vitamin D (I.C.U.)	100
Riboflavin, mg.	1.8
Pantothenic acid, mg.	5.0
Niacin, mg.	25
Pyridoxine, mg.	1.2

* Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1954.

[1] These figures are estimates of requirements and include no margins of safety.

TABLE A–12. Average Composition of Some Poultry Feeds*

Feeding Stuff	Moisture, %	Protein, %	Fat, %	Fiber, %	N-free Extract, %	Ash, %	Calcium, %	Phosphorus, %	Iron, %	Manganese, mg./lb.	Copper, mg./lb.	Cobalt, mg./lb.	Carotene, mg./lb.	Thiamine, mg./lb.	Niacin, mg./lb.	Riboflavin, mg./lb.	Pantothenic Acid, mg./lb.
Alfalfa meal, dehydrated, 17% protein	6.7	17.8	2.8	24.2	39.7	8.8	1.7	0.2	0.033	15.0	3.1	0.05	36.0	1.5	8.7	7.3	12.3
Alfalfa meal, sun-cured, 17% protein	7.8	17.6	2.0	23.8	38.9	9.9	1.4	0.2	...	...	...	...	24.0	1.1	16.1	5.0	12.7
Alfalfa meal, dehydrated, 20% protein	7.1	20.9	2.9	19.8	38.2	11.1	1.7	0.3	0.039	28.6	7.1	0.17	60.0	3.1	17.3	7.4	18.5
Alfalfa meal, sun-cured, 20% protein	8.1	20.3	3.2	17.8	40.3	10.3	1.4	0.2	...	...	...	...	48.0	2.0	22.7	7.2	13.6
Barley, excluding Pacific Coast	10.6	12.7	1.9	5.4	66.6	2.8	0.09	8.5	0.005	8.3	5.1	0.01	...	1.7	24.1	0.8	3.7
Barley, Pacific Coast	11.0	9.7	2.2	6.2	68.7	2.2	0.06	0.4	0.007	7.8	5.0	...	1.8	1.8	20.0	0.6	3.3
Bone meal, raw	6.4	26.0	5.0	1.0	2.5	59.1	21.7	10.0	0.044	1.9	8.5	0.05	...	0.1	1.9	0.5	1.0
Bone meal, steamed, special	3.3	13.4	9.7	1.1	1.2	71.3	29.3	15.1	0.080	5.1	9.0	0.03	...	0.9	2.0	0.4	0.8
Buttermilk, dried	7.6	32.4	6.4	0.3	43.3	10.0	1.4	0.9	...	1.5	...	...	...	1.7	2.8	15.8	13.5
Corn, dent, yellow	15.0	8.9	3.9	2.0	68.9	1.3	0.02	0.3	0.002	2.3	0.9	0.01	1.33	1.7	9.8	0.5	2.6
Corn gluten meal, 41% protein	8.6	42.9	2.0	3.9	40.1	2.5	0.2	0.4	0.047	4.4	13.1	0.04	10.0	0.1	24.8	0.7	3.8
Cottonseed oil meal, 41% protein	7.8	41.2	6.2	11.2	27.7	5.9	0.2	1.2	0.008	12.9	7.7	0.06	...	1.8	13.0	2.5	4.4
Crab meal	7.9	31.5	2.2	10.5	6.0	41.9	14.5	1.5	...	...	...	...	...	...	...	2.3	3.0
Distiller's dried corn grains, with solubles	6.9	28.8	8.9	9.0	41.7	4.7	0.2	0.7		18.2	...	...	...	2.1	36.3	3.4	5.2
Distiller's dried solubles	6.9	28.0	6.7	3.3	47.6	7.5	0.4	1.4	0.050	45.4	36.3	0.09	...	2.7	54.3	5.2	8.9
Fish meal, menhaden	6.4	62.2	8.5	0.7	4.2	18.0	5.0	3.4	0.057	1.0	3.9	0.08	...	0.2	25.9	24.4	...
Fish meal, sardine	6.9	67.2	5.0	0.6	5.4	14.9	4.2	2.5	0.03	10.3	9.2	0.08	...	0.2	26.0	2.5	1.3
Fish meal, whitefish	9.6	63.0	6.7	0.1	0.1	20.5	6.8	3.7		5.0	...	...	...	0.4	36.0	4.0	1.4
Hominy feed, white, 5% fat	10.1	10.8	5.7	4.7	66.0	2.7	0.05	1.0	0.004	8.2	0.9	...	...	5.9	25.1	1.0	3.1
Hominy feed, yellow	9.5	11.1	7.1	5.8	63.9	2.6	0.05	0.7	0.024	6.9	5.7	0.02	0.5	3.8	22.6	1.0	3.5
Limestone	...	...	...	...	...	...	38.3	...	...	...	...	...	...	...	...	...	...
Linseed oil meal, o. p., 33% protein	8.8	35.0	5.6	8.1	36.9	5.6	0.4	0.9	0.024	19.1	11.8	0.17	...	3.9	18.9	1.9	7.5
Linseed oil meal, o. p., 37% protein	9.1	38.0	5.9	7.7	33.7	5.6	0.5	0.9	0.028	18.6	11.9	0.18	...	2.7	14.7	1.7	8.9
Meat scrap, 52% protein	6.9	52.9	7.3	2.2	4.3	26.4	8.7	4.4	0.050	4.0	5.5	0.09	...	0.1	27.1	2.4	2.1
Meat scrap, 60% protein	6.2	60.9	8.8	2.4	1.1	20.6	6.3	3.5	0.041	5.4	3.7	0.04	...	0.1	23.7	2.5	2.3
Meat and bone scrap, 50% protein	6.4	50.6	10.0	2.0	2.0	29.0	9.7	4.2		5.3	...	...	...	...	21.4	2.1	1.5
Milk, skimmed, dried	5.8	34.7	1.2	0.2	50.3	7.8	1.3	1.1	0.005	1.2	5.2	0.03	...	1.5	5.7	10.0	16.0
Molasses, beet	19.5	8.4	0.0	0.0	62.0	10.1	0.08	0.02	...	...	...	...	...	...	22.0	...	...
Molasses, cane	26.0	2.9	0.0	0.0	62.1	9.0	0.7	0.08		...		...	...	0.4	20.9	1.1	17.9
Oats, excluding Pacific Coast	9.8	12.0	4.6	11.0	58.6	4.0	0.09	0.4	0.008	19.2	2.4	...	...	2.9	8.2	0.4	6.8
Oats, Pacific Coast	9.8	9.0	5.4	11.0	61.1	3.7	...	...		...	...	...	...	...	...	...	...
Oatmeal, feeding	9.3	15.0	7.4	2.0	64.4	1.9	0.07	0.4	0.006	13.6	1.6	0.02	...	3.5	4.5	0.6	6.6
Oyster shell, ground	...	...	...	...	...	...	37.9	...	...	...	...	...	...	...	...	...	...

Peanut oil meal, o. p., 43% protein	7.2	43.1	7.6	13.9	23.0	5.2	0.1	0.6	...	...	...	...	...	3.3	77.5	2.4	24.1
Phosphate, defluorinated rock	...	...	...	...	...	...	28.3	12.3	...	40.9	29.5	...	...	...	...	.	...
Phosphate, dicalcium	...	...	...	...	...	...	26.5	20.5	...	...	...	...	...	...	...		...
Rice bran	9.0	12.8	13.1	12.7	41.7	10.7	0.08	1.4	...	70.9	...	...	...	10.3	129.1	1.4	10.3
Rice polishings	9.7	12.7	11.4	3.5	56.6	6.2	0.04	1.1	...	...	...	...	...	8.8	325.0	0.9	5.5
Rye, grain	10.5	12.6	1.7	2.4	70.9	1.9	0.01	0.3	0.008	37.0	3.4	...	...	2.0	7.1	0.7	4.2
Sorghum, kafir	10.5	11.2	2.9	2.3	71.1	2.0	0.04	0.3	0.008	7.5	3.0	0.03	...	1.6	18.3	0.5	5.7
Sorghum, milo	10.6	11.3	2.9	2.2	71.3	1.7	0.03	0.3	0.005	5.9	7.8	0.02	...	1.8	13.1	0.4	5.0
Sorghum, milo head chop	10.0	10.1	2.5	6.8	67.1	3.5	0.1	0.3	...	...	...	...	...	...	...	0.9	...
Soybean oil meal, 41% protein	10.2	41.1	5.3	5.5	30.4	7.5	0.3	0.6	0.017	12.3	11.1	...	...	...	...	2.5	...
Soybean oil meal, 43% protein	9.4	43.1	5.6	5.7	30.4	5.8	0.3	0.6	...	12.7	...	...	...	...	...	1.5	...
Soybean oil meal, 44% protein	9.2	44.2	5.3	5.6	29.9	5.8	0.3	0.7	0.015	14.0	7.7	0.05	...	0.8	16.7	2.0	6.1
Soybean oil meal, solvent, ext.	9.4	46.1	1.0	5.9	31.8	5.8	0.3	0.6	0.01	13.8	6.8	...	...	1.4	17.1	1.4	6.2
Wheat, hard red winter	10.4	15.2	1.8	2.6	68.3	1.7	0.05	0.4	0.005	18.0	2.0	0.03	...	2.3	24.1	0.5	6.3
Wheat, northern spring	9.9	15.8	2.2	2.5	67.8	1.8	0.05	0.4	0.005	33.0	4.9	...	...	2.3	28.8	0.5	6.4
Wheat, soft, Pacific Coast	10.8	9.9	2.0	2.7	72.7	1.9		0.3	0.007	27.7	4.4	...	...	2.2	26.8	0.5	5.2
Wheat bran	10.3	16.4	4.3	9.9	53.0	6.1	0.1	1.3	0.016	56.0	4.9	0.05	...	3.9	63.5	1.4	13.6
Wheat flour middlings	10.3	18.1	4.6	4.9	58.5	3.6	0.07	0.6	0.002	39.0	2.1	...	...	6.0	44.2	0.8	4.5
Wheat standard middlings	9.9	17.6	5.0	6.7	56.6	4.2	0.1	0.8	0.009	53.6	9.8	0.04	...	5.8	44.3	0.8	9.3
Whey, dried	6.5	12.2	0.8	0.2	70.4	9.9	0.9	0.8	0.02	1.1	24.2	0.05	...	1.8	5.1	13.0	22.4
Yeast, brewer's, dried	6.3	46.8	1.2	2.8	35.7	7.2	0.1	1.5	0.014	2.4	15.1	0.08	...	43.0	213.6	14.0	49.1

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* Data on the average composition of feedstuffs were supplied by the Committee on Feed Composition of the National Research Council. See: Nutrient Requirements for Poultry, *Publication* 301, Committee on Animal Nutrition, National Academy of Sciences, National Research Council, revised 1954.

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An italic number designates a page on which a major reference to a topic may be found.

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