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Biochemical Bulletin

Edited, for the Columbia University Biochemical Association, by

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ANNOUNCEMENTS

Future issues of the Biochemical Bulletin

Pursuant to a further change of plan, which is referred to editorially on page 270, this (March) number is the first issue of Volume IV of the BIOCHEMICAL BULLETIN. Hereafter, the volumes of the BIOCHEMICAL BULLETIN will coincide, in periodicity, with the calendar years, instead of the academic years as heretofore. The new plan will enable us to issue the quarterly numbers promptly hereafter.—in March, June, September and December.

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F. H. Storer

BIOCHEMICAL BULLETIN

VOLUME IV

MARCH, 1915

No. 13

IN MEMORIAM

FRANCIS HUMPHREYS STORER

Born, March 27, 1832 Died, July 30, 1914

With the demise of Professor Francis Humphreys Storer, Professor in the Bussey Institution of Harvard University, on July 30th last, at the age of 82 years, there ended a long and useful career devoted principally to chemistry in its relation to agriculture. Frank Storer, as he was known in the early days, was born on Boylston Street, Boston, and was the son of David Humphreys Storer, M.D., LL.D., and Abby Jane (Brewer) Storer. He received his early training in private schools and under private tutors, and from 1850 to 1851 was a student at the Lawrence Scientific School of Harvard University. From 1851 to 1853 he served as assistant to Professor Josiah P. Cooke, then Professor of Chemistry at Harvard. In 1853 he was chemist with the U. S. North Pacific Exploring Expedition. After his return to Massachusetts, Storer resumed his studies at the Lawrence Scientific School and graduated with the degree of Bachelor of Science in 1855.

Although not reared in an agricultural community, Storer was a profound lover of nature and in his younger days, it is said, he seized every opportunity to visit the countryside. Endowed with a keen imagination, he was one of the few to realize the need of a better basis for the practice of farming on the American continent. Rule of thumb methods prevailed at the time to the extreme, and in many cases where crops were successfully grown, or where not,

the outcome was attributed to this or that cause, but hardly ever to the chemical factors operating in the soil.

Impressed with the idea of the need of placing agriculture on a plane with the other sciences, Storer went abroad in 1855 to study the European methods of applying chemistry to the study and practice of agriculture. At Tharand he is found working in the laboratory of the Royal Academy of Agriculture, studying methods under the famous Julius A. Stöckhardt. At Heidelberg he listened to the lectures of the great Robert Wilhelm Bunsen, and last, but not least, we hear of him making observations in Paris under the master Boussingault.

Not finding an appropriate opportunity for applying his newly-gained knowledge—the application of chemistry to the interpretation of biological processes—Storer, on his return to the United States in 1857, while the “panic of 1857” was at its height, established himself as a consulting and analytical chemist in Boston. In 1865, however, he accepted a position as chemist with the Boston Gas Light Company, and also became Professor of General and Industrial Chemistry at the newly-created Massachusetts Institute of Technology. This was Professor Storer’s first real experience as an independent teacher and here he taught chemistry, as he often remarked later, “better than ever before in America.”

Prof. William B. Rogers, the Founder of the Massachusetts Institute of Technology, was strongly of opinion that the right way to teach the sciences—chemistry, physics, and biology—was the laboratory way, without rejecting entirely the lecture method, in which he was himself a master. He insisted when he started scientific instruction in the Institute of Technology, that every student should have abundant opportunity to make experiments himself in properly equipped laboratories. The first man he selected to be Professor of Chemistry in the new Institute was Storer, with whose thorough laboratory training in chemistry, and experience as a practicing chemist, Professor Rogers was familiar. Professor Rogers had also known about some chemical researches Storer and Charles W. Eliot had made together in the early '60s, especially with one published as a memoir in the series of the American Academy of Arts and Sciences on “The impurities of commercial zinc.” Professor

Rogers knew that Eliot also believed in the laboratory method of teaching chemistry.

After the selection of Professor Storer had been made, Eliot received, at Vienna, a letter from Professor Rogers, asking Eliot to become professor of analytical chemistry in the Institute, and telling him that his friend Storer was to be the other professor of chemistry.

Storer and Eliot began, in September, 1865, to teach chemistry by the laboratory method to the first class enrolled in the Massachusetts Institute of Technology in a small and poorly equipped room on the second story of a mercantile building on Summer Street, nearly opposite the store of C. F. Hovey & Co. But Rogers Hall was nearly finished; and in the course of that year President Rogers assigned the rooms in the new building to the Chemical Department, and provided the money with which to furnish and equip the laboratories. Storer and Eliot made all the detailed plans, and supervised the constructions on the principle that in all chemical subjects every student was to have desk-room and apparatus for conducting experiments several hours a week with his own eyes and hands.

Foreseeing the need of laboratory manuals, first in general chemistry, and then in qualitative analysis, Storer and Eliot soon began the preparation of these books,—first the “Manual for Inorganic Chemistry,” and then the “Manual for Qualitative Analysis.” These books were written in a manner then novel, though now familiar—some chapters by Storer and some by Eliot. The manuscript having been put into type, the authors used the proofs in their classes for one year in the Institute laboratories, and in this process discovered and remedied some defects, and made many improvements. It is related, by one who knew of the relations existing between these pioneer teachers of chemistry, that when it came to publishing the book, a title page was demanded of them; and each author maintained that the other’s name ought to stand first. Discussion led to no result; so they tossed up a cent to decide the question by chance. Storer picked up the cent, and announced that Eliot’s name was to stand first. Eliot accused him of not having looked at the cent; but he would not recognize the correctness of Eliot’s observation. So the book became known as Eliot and Storer’s; but the authors succeeded in putting on the back of the book the names

Eliot and Storer crossed—one on top of the other—as an indication that the order of the title page had no real meaning.

The “Manual of Inorganic Chemistry” sold in considerable numbers for a long term of years, and is still in the market after several revisions. It was at first the only book of the kind in the English language; and, indeed, there was no equivalent in any language; but within a few years many manuals appeared which were intended to promote the same laboratory method of instruction in chemistry. A few years after its first appearance, one of the authors was one day visiting Rugby School in England, and found that the Master who taught chemistry at that famous School was using it in the laboratory which he had set up for the teaching of chemistry. He accounted for the presence of this American text-book in the School by frankly saying that he had not been able to find an English book which answered the same purpose, or was conceived in the same spirit. In 1869 Eliot became President of Harvard University, and thereafter Storer made all the revisions of the two manuals he and Eliot had written together.¹

Judging from his “Cyclopedia of Quantitative Chemical Analysis,” prepared during his stay at the Massachusetts Institute of Technology, it is evident that the mind of Professor Storer was an extremely practical one. This book is a silent witness to the work of a pioneer, and from the preface we note that the object of writing it was “not only to provide the student and working chemist with a comprehensive dictionary of quantitative processes, but to call the attention of the chemical fraternity to the question of the possibility of presenting this branch of chemical art in a more serviceable and manageable form than has been customary hitherto. The experiment is certainly worth the trying whether a definite system of classifying substances in alphabetical order, and of referring each and every process to the fundamental fact or principle upon which it depends, will not greatly facilitate both the study and the practice of analysis. . . . The tendency of all the works recently published (1869) on quantitative analysis is towards condensation and ab-

¹ Dr. Charles W. Eliot was Professor of Analytical Chemistry and Metallurgy at the Massachusetts Institute of Technology from 1865 to 1869, and from then on, President of Harvard University. He was Professor Storer's brother-in-law.

breiviation, while the aim of the present book is to show that perspicuity can be best gained by amplification, if need be, and methodical arrangement."

Thus, for example, in the case of aluminum acetate we find, first, the principles (underlying the method); second, applications (of the method); third, the various methods; and fourth, the precautions (to be observed). Truly a noble viewpoint, and one which undoubtedly called for many sacrifices.

As a bibliographer Storer had few equals. One is especially impressed with this fact when examining "The First Outlines of a Dictionary of Solubilities," prefaced in 1862 and published in 1864. This work, probably the only one of its kind in the English language at the time, and today still a veritable mine of information, surely was a labor of love, and a monument to one who deemed it a pleasure to lessen the burden of others.

When the Bussey Institution, a School of Agriculture and Horticulture, was finally organized in 1870, Francis Humphreys Storer was chosen on November 25, 1870, to be its Professor of Agricultural Chemistry, and in 1871 he became Dean. In this capacity Storer was at his best and it marked the beginning of an era of much fruitful and fundamental agricultural research. The founding of the Bussey Institution was, to Storer, "the nearest thing to an agricultural experiment station in Massachusetts."

The status of Professor S. W. Johnson of the Sheffield Scientific School of Yale University, was very similar to that of Professor Storer at Harvard. Many of the ideas of the two savants were alike.² Thus in 1878, under date of April 26, Storer writes: "I noted (even before you wrote) what you say of Miller's cows vs. meal. 'Tis just what I would have said myself." Again, in a letter dated April 3, 1880, to Samuel W. Johnson, we note the following: "I am glad you hold your 'luff' in respect to the conventional method of stating analyses of fodder. There is no sense in trying to refine this thing beyond the possibly practical. We are hardly more ripe than Einhof and Sprengel were for the complete analysis of rough fodders, and there is a semblance of—(let us say

² See "From the Letter Files of S. W. Johnson," by Elizabeth A. Osborne, Yale University Press, 1913.

ignorance) in holding up the names of too many chemicals to the gaze of the great and unsoaked public. It is bad enough to have to report the 'fat' of hay as if it were really oil. What we really need is a critical sifting of all the analyses with the view of discovering the best possible means, in the light of existing knowledge. The question is one of chemistry far more than of arithmetic. There are manifold instances of 'maxima' and 'minima' which could be thrown out at once, for cause."

The first results of Storer's labors were published in 1874, which was during pre-experiment station (federal) days, in the *Bussey Bulletin*. The first bulletin emanating from an experiment station in the United States was published in August 1877, by Samuel W. Johnson. The first *Bussey Bulletin* was entitled "A report of the results obtained on examining some commercial fertilizers, by way of analysis," by F. H. Storer (in 1874).

The analyses reported were incidental to field experiments undertaken by the Bussey Institution in behalf of the trustees of the Massachusetts Society for Promoting Agriculture. The field tests were made for the purpose of testing the efficiency of a variety of substances sold in Boston and supposed to possess fertilizing power. The early bulletins on agricultural subjects are not mere reports of analyses, but worthy discussions of the topics and they are easily comparable with the ones issued by the best agricultural experiment stations of today. Thus in *Bussey Bulletin* No. 2 we find the following: "As regards the item 'cellulose,' for example, the table shows conclusively that while hay contains 30 percent of woody fiber, so compact that it can withstand the tolerably long-continued action of dilute acid and alkali; that while oats contain 10 and one-third per cent, brewers' grains 6.2 per cent (in a total of only 22 and one-quarter per cent of dry organic matter), and dry whiteweed 31 per cent of this resisting substance, bran yields no more than eight and one-third per cent of it when exposed to precisely similar treatment, and maize only about three per cent. The method ordinarily used for determining cellulose is undoubtedly far from being perfect, as I may have occasion to show in a future communication."

Professor Storer had a profound respect for the work done by

others. This fact is indelibly recorded in the *Bussey Bulletins*, which, in almost all cases contain an account of the literature pertaining to the subject under discussion. The work of the Bussey Institution included some of the earliest well-planned and systematic experiments on the field valuation of fertilizers, and in which chemical analysis played an important but subordinate part. This is clearly shown by the results reported in Bulletin No. 7, entitled "A record of trials of various fertilizers upon the plain-field of the Bussey Institution." This third report gives the results obtained in 1873 and also reviews the three-year course of experiments.

I doubt very much if the *Bulletins* of the Bussey Institution have been read or consulted to the extent that they should have been by agriculturists—they contain much that affects agricultural conditions today.

The financial condition of the Bussey Institution was affected considerably by the Boston fire and the financial crisis in 1873. From these inroads into the Bussey fund the Institution never recovered. Despite these setbacks, Professor Storer kept on with his investigations and teaching, often receiving no salary for his services, or an amount so small that it was in no way commensurate with his ability and the services he rendered. To what extent Professor Storer's financial condition suffered by his interest in agriculture I am unable to say, but a reply to a letter sent to Storer by Professor Johnson³ from New Haven, Conn., December 15, 1879, may give us some light on the subject.

"NEW HAVEN, CT., Dec. 15, 1879.

My dear Storer: I am most profoundly sorry at the state of Bussey in general and . . . in particular. As to the questions—I only know what I got or rather I know nothing beyond that. I can't certainly say whether it was \$35 per column that I first worked for, for the Tribune, or not, but I think it was that. I struck for \$50 per column, had it for 6 months, then declined to go on. . . . I shall at once see if I can't suggest to some good parties that they may get you to write for their papers, etc.

Yours most faithfully."

³ See footnote, page 5.

The Bussey Institution closed its doors in 1907 to those for whom it was intended, *i. e.*, first, young men who intended to become practical farmers, gardeners, florists, or landscape gardeners; second, young men who would naturally be called upon to manage large estates or who would make good stewards or overseers of gentlemen's estates; and third, persons who wished to study some special branch of agriculture, horticulture, botany, or applied zoology.

Professor Storer's three-volume work on "Agriculture in some of its relations with chemistry" was probably the crowning success of his literary and agricultural career. It has passed through seven editions. The esteem in which this authentic treatise was held after its issue can best be gleaned from the following statement taken from the Harvard University Report of 1889, "The work combines very happily the statement of scientific principles with due regard for financial and other practical considerations; and it is written in an easy, popular style that should render its perusal most pleasurable for any intelligent agriculturist, however slight his acquaintance with chemical terminology. . . . His new work is a splendid contribution to agricultural science, is in fact almost monumental in character, and it must be many years before it can possibly be superseded by anything better."

One who knew him intimately has said that all his life Storer was an omnivorous reader; and as he had a very retentive memory and an unusual alertness and vivacity in conversation, he was a very instructive and inspiring companion for his intimates, of whom, however, there were but few. He gradually ceased to attend the scientific societies of which he was a member, withdrew more and more from society, and lived in his books, and in the circle of his immediate relatives. His habits were always simple and abstemious; so that he lived to be eighty-two years of age with unimpaired mental interests and powers, though with some bodily infirmities and limitations. His conscience was quick, his intelligence keen and rapid, and his temperament sensitive and impetuous, but not sanguine and serene enough for steady happiness. As a man of science he was spotless—a candid, devout, lover and seeker of truth.

I can not but think that too little has been made of Professor Storer's scientific services to agriculture. Of the multitude who know nothing of his work this was not to be expected, but from the American agriculturist, plant physiologist, agricultural chemist, etc., it can command nothing but gratitude and respect.

At the April meeting in 1907 of the President and Fellows of Harvard College, the resignation of Francis Humphreys Storer as Professor of Agricultural Chemistry of Harvard University, and Dean of the Bussey Institution, was accepted. The minutes of that meeting on the services of Professor Storer are as follows:

"The services of Professor Storer to the Bussey Institution began with his appointment to the Professorship of Agricultural Chemistry on November 25, 1870, and have continued without any intermission to the present day. They comprehended stated teaching in the lecture room and laboratory; the production of a comprehensive and durable treatise on Agricultural Chemistry; and the general administration of the Institution, including its library and *Bulletin*. As a teacher, Professor Storer was highly interesting and helpful, because of his wide range of knowledge and his wealth of illustrative material. As an administrator, he was diligent, frugal in expenditure, and especially sympathetic with students whose means and attainments were limited, and whose early opportunities had been few. He devoted himself without reserve to the Bussey Institution in spite of the fact that the Boston fire in 1872 greatly and permanently reduced its resources and changed its prospects."

LEWIS WILLIAM FETZER.

*Office of Expt. Stations, U. S. Dep't of
Agriculture, and Georgetown University,
Washington, D. C.*

PUBLICATIONS OF FRANCIS HUMPHREYS STORER

Bulletins of the Bussey Institution

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L. W. F.

* These publications have passed through several editions.

ON THE BEHAVIOR OF KERATIN SULFUR AND
CYSTIN SULFUR, IN THE OXIDATION OF
THESE PROTEINS BY POTAS-
SIUM PERMANGANATE. I*

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Introduction. Previous researches on the products formed from proteins in general and from keratin in particular, by the action of potassium permanganate, give almost no indication of the fate of the sulfur in this oxidation process. Since it is now known, as a result of Maly's¹ investigations, that the total sulfur of egg-white remains in oxy-proto sulfonic acid, during permanganate oxidation, it is natural to ask: how does the sulfur of keratin, and of other sulfur-yielding albuminoids, behave in such oxidation? Following a suggestion of Prof. Dr. VI. Gulewitsch, I have undertaken the oxidation of human hair and of cystin, and have investigated the oxidation-products in relation to the sulfur content.

Experimental. A. First I determined the sulfur-content of dry, fat-free, human hair.

I. 0.4106 gm. of human hair was fused with a mixture (1:8) of potassium hydroxid and potassium nitrate, over a small alcohol flame. The fused mass was dissolved in water; the solution was acidified with hydrochloric acid, after the addition of a few drops of bromin water, and evaporated to dryness on a water-bath; the residue was dissolved in water, filtered, precipitated in the usual way with barium chlorid, and the precipitate ignited and weighed. The amount of barium sulfate obtained was 0.1661 gm. (0.02282 gm. sulfur). The hair contained, therefore, 5.56 percent of sulfur.

Then the sulfur-content of the oxy-proto sulfonic acid derived from the hair was determined. For this purpose I took 200 gm. of

* Translated from the author's manuscript, in German, by Dr. Edgar G. MiHer, Jr.

¹ Maly: *Sitzungber. d. k. Acad. d. Wiss.*, 1885, xci (II Abteil), p. 157.

fat-free human hair, and digested it with 6 l. of 2 percent sol. of potassium permanganate. The mixture was allowed to stand for several days, with occasional shaking. The clear fluid was filtered, and the filtrate acidified with hydrochloric acid. The resulting precipitate was washed, first by decantation and then on a filter, redissolved in dilute soda sol. and precipitated by acidification. The precipitate was thoroughly washed, and dried, first in the air, then in an air-bath at 110° C. The dry substance was weighed and analyzed in the manner described above.

II. 1.3399 gm. of the substance gave 0.1407 gm. of barium sulfate (0.01933 gm. of sulfur).

III. 0.1900 gm. yielded 0.1980 gm. of barium sulfate (0.00272 gm. of sulphur).

The substance contained, therefore, judging from the results of the two analyses, 1.44 percent and 1.43 percent of sulfur, respectively. The oxy-proto sulfonic acid from egg-white contains, according to Maly,² 1.77 percent of sulfur. Since only a small amount of oxy-proto sulfonic acid resulted from the oxidation of the hair, it is clear that only a small part of the sulfur of the keratin remained in the oxy-proto sulfonic acid.

In order to determine how much sulfur is held in the solution as sulfuric acid, and how much is held in the form of some organic combination, after oxidation by permanganate, I have carried out two parallel experiments on the products of the oxidation.

In the *first experiment*, 9.2160 gm. of dry, fat-free hair were digested in 700 c.c. of 2 percent sol. of potassium permanganate. After four days the liquid over the hair was perfectly clear. It was filtered, and from it two portions of 100 c.c. each were taken (IV — V).

IV. The first *portion* was evaporated to dryness, treated with dilute hydrochloric acid, and the resultant precipitate of oxy-proto sulfonic acid was repeatedly extracted with water; the filtrate, together with the wash-water, was evaporated, and precipitated with barium chlorid. It yielded 0.040 gm. of barium sulfate (0.0055 gm. of sulfur). Therefore, in the 700 c.c., 0.0385 gm. of sulfur was held as sulfuric acid.

² Maly: *Monatsch. f. Chemie*, 1884, viii, p. 255.

V. The second *portion* of the same fluid was evaporated to dryness, and the sulfur-content determined, after fusion with potassium hydroxid and potassium nitrate. The result was: 0.5172 gm. of barium sulfate (0.07105 gm. of sulfur). Hence, the sulfur-content of 700 c.c. was 0.4974 gm. It is clear that a much greater amount of the sulfur (92.3 percent) is contained in the filtrate from the oxy-proto sulfonic acid in the form of some organic combination, and only a small part (7.7 percent) is present in the solution as sulfuric acid.

For the *second experiment*, 10.2085 gm. of dry fat-free human hair were digested with 800 c.c. of 2 percent sol. of potassium permanganate and allowed to stand for 3-4 days, until the fluid was perfectly clear. This was then filtered and, of the filtrate, two portions of 100 c.c. were taken.

VI. The first *portion* was treated as in determination IV, with the difference, that this time the sulfur-content of the oxy-proto sulfonic acid precipitate was also determined. The filtrate and wash-water were treated with barium chlorid after the removal of the oxy-proto sulfonic acid, and yielded 0.4230 gm. of barium sulfate (0.005811 gm. of sulfur). Hence, in 800 c.c., 0.0465 gm. of sulfur was present as sulfuric acid.

VII. After the fusion of the oxy-proto sulfonic acid precipitates, with a mixture of potassium hydroxid and potassium nitrate, 0.0117 gm. of barium sulfate (0.00161 gm. of sulfur) was found. The sulfur in the oxy-proto sulfonic acid amounted, therefore, to 0.0129 gm.

VIII. The second *portion* was treated as in determination V. After fusion, 0.4694 gm. of barium sulfate (0.06449 gm. of sulfur) was obtained. Hence, 0.5159 gm. of sulfur was present in 800 c.c.

From these experiments it follows, unquestionably, that the greater part of the sulfur occurs, after oxidation with permanganate, as water-soluble organic substance. Of the total sulfur-content in the dissolved oxidation products (0.5159 gm. of sulfur) there were found: 9 percent as sulfuric acid, 2.5 percent as oxy-proto sulfonic acid, and 88.5 percent as a water-soluble organic substance.

This water-soluble substance gives a precipitate with lead ace-

tate, dissolves slightly in dilute alcohol, and is almost insoluble in 95 percent alcohol. I have used the following method, based on these properties, for the isolation of this substance: 70 gm. of hair were digested with 5-6 l. of 2 percent potassium permanganate sol. and allowed to stand for several days. The clear fluid was filtered, acidified with hydrochloric acid, and the precipitate separated by filtration. The filtrate was made slightly alkaline with dilute soda sol., and precipitated with lead acetate. The precipitate was washed, first by decantation and then on a filter, suspended in water, and treated with hydrogen sulfid. Precipitated lead sulfid was removed by filtration, the filtrate evaporated to a syrupy consistence, and extracted several times with 95 per cent alcohol. The residue was dissolved in a small amount of water, and the solution yielded a precipitate with a large excess of alcohol. This precipitate was dissolved in a little water and re-precipitated with alcohol. The final product contained 12.5 percent of ash and 8.81 percent of sulfur. In order to purify the product it was re-dissolved several times in water, re-precipitated with alcohol, and washed with alcohol and ether. This purified material was dried at 110° and analyzed (IX-XVI).

IX. 0.1437 gm. gave, after ignition, 0.0089 gm. of ash, which consisted of silicic acid, with traces of potassium, sodium, calcium, iron and manganese.

X. 0.0743 gm. gave, after ignition, 0.0046 gm. of ash.

XI. 0.1881 gm. gave, after burning with lead chromate, 0.2537 gm. of carbon dioxide and 0.0928 gm. of water.

XII. 0.1808 gm. burnt with lead chromate, yielded 0.2385 gm. of carbon dioxide and 0.0908 gm. of water.

XIII. 0.2166 gm. gave 26.3 c.c. of nitrogen, at 23.5° and 763 mm. Hg.

XIV. 0.1762 gm. gave 21.1 c.c. of nitrogen, at 22.5° and 762 mm. Hg.

XV. 0.1578 gm. gave, on fusion by the method described above (determination I), 0.1121 gm. of barium sulfate.

XVI. 0.1787 gm. gave 0.1247 gm. barium sulfate.

Summary: Found (percent)

	IX	X	XI	XII	XIII	XIV	XV	XVI	Average percent
C	—	—	36.78	35.98	—	—	—	—	36.4
H	—	—	5.52	5.62	—	—	—	—	5.6
N	—	—	—	—	13.70	13.57	—	—	13.6
S	—	—	—	—	—	—	9.76	9.59	9.7
O	—	—	—	—	—	—	—	—	28.5
Ash	6.19	6.19	—	—	—	—	—	—	6.2

Summary: Calculated.

	Ash-free subst. percent	$C_{10}H_{17}N_3SO_6$ percent	$C_{30}H_{54}N_{10}S_2O_{13}$ percent
C	38.8	39.1	38.3
H	5.9	5.6	5.8
N	14.5	13.7	14.9
S	10.3	10.4	10.2
O	30.5	31.2	30.8
Ash	—	—	—

The substance so obtained is acid in reaction, is very hygroscopic, and gives, with lead acetate, a precipitate which is soluble in an excess of the reagent, and in lead acetate sol. The substance gives the biuret reaction. Its aqueous solution gives, on treatment with alcohol, a milky turbidity which, on evaporation, deposits small crystals. The material is very stable and, on heating with mineral acids, is decomposed with the formation of sulfuric acid. To determine its basicity the aqueous sol. was titrated with 0.0983/*N* soda sol., with lacmoid-naphthol-green as the indicator.

XVII. 1.3 c.c. of the soda sol. was required to neutralize 0.1053 gm. of the substance dissolved in water. For a monobasic acid ($C_{10}H_{17}N_3SO_6$)₃, 1.2 c.c. would be necessary, but it must be considered that the ash of the substance contained a large amount of silicic acid.

B. In order to ascertain the sulfur-distribution among the oxidation products of *cystin*, I have oxidized, with potassium permanganate, cystin prepared from human hair. About 2.3 gm. of cystin, containing 0.61 gm. of sulfur, were oxidized with 1 l. of permanganate sol. All of the filtrate, together with the washings, was evaporated and the contained sulfuric acid determined (XVIII).

XVIII. I obtained 2.0931 gm. of barium sulfate (0.2875 gm. of sulfur). Therefore, 47 percent of the total sulfur was changed to sulfuric acid in the oxidation.

To 40 c.c. of the filtrate, which was obtained in the oxidation of the hair with 2 percent potassium permanganate sol. (p. 19) and which contained 0.0022 gm. of sulfur as sulfuric acid, was added 0.2300 gm. of cystin (with a sulfur-content of 0.0614 gm.), and 45–50 c.c. of 2 percent permanganate sol. After the reaction was ended, the fluid was filtered, the precipitate washed and the filtrate together with the washings was evaporated to dryness. The residue was acidified with hydrochloric acid, and the precipitate of oxy-*proto* sulfonic acid separated by filtration. The new filtrate, with the washings, was then precipitated with barium chlorid in the usual way (XIX).

XIX. From this precipitate 0.2266 gm. of barium sulfate (0.0311 gm. of sulfur) was obtained. Thus, from the oxidation of cystin I obtained 0.0311–0.0022, 0.0289 gm. of sulfur, or 46.9 percent of the total sulfur, as sulfuric acid.

From the results of these experiments it follows that a much larger amount of sulfur is split off as sulfuric acid by the oxidation of cystin with permanganate than by the similar oxidation of keratin. It is further to be noted that, in the process, some cystin always remains unoxidized and that, among the oxidation products of cystin, a small amount of hydrogen sulfid is always present, while in the distillates of the oxidation products of hair, hydrogen sulfid is never found.

General conclusions. These data suggest the following conclusions: In the oxidation of *keratin* the largest part of the contained sulfur remains in organic combination, and only one tenth is converted into sulfuric acid. The oxidations of *cystin* and of keratin proceed along different lines.

I have undertaken further work on this subject.

SERUM DIAGNOSIS OF ROUS'S CHICKEN SARCOMA, BASED ON CHEMICAL METHODS

CASIMIR FUNK

Introduction. Serum diagnosis of tumors is, at present, in a preliminary stage. During the last few years there have been described several methods which are mostly based on biological properties of serum. None of these methods, however, has given satisfactory results. Among the procedures tried by the author were the method of Freund, the meiostagmin-reaction of Ascoli, and the optical method of Abderhalden. In view of the failure of these biological tests to give a reliable method for tumor diagnosis, it seemed advisable to ascertain whether purely chemical methods would serve better for this purpose.

This investigation was conducted with serum of chickens inoculated with Rous's chicken sarcoma. The large amount of serum obtainable from these animals was a great advantage for our preliminary studies. The tumor used being very malignant, the animals died in a few weeks. One would therefore expect the chemical changes to have been very pronounced. In the first part of the work, the serum was precipitated with absolute alcohol; and the amount of precipitate, and its nitrogen and phosphorus contents, were estimated. The filtrate was analyzed in the same way. Throughout the whole inquiry, normal and tumor sera were treated simultaneously. The results tend to show that the tumor serum was poorer in proteins and phosphorus.

Better results were obtained by analyzing serum itself. In this case the proportions of nitrogen, phosphorus, sulfur, chlorine, amino-nitrogen, and sugar, and the molecular depression, were taken into account. Of 22 tumor sera, 20 gave practically identical results. Tumor serum was, as a rule, poorer in nitrogen, phosphorus and sulfur than normal, though richer in amino-nitrogen; the molecular depression was greater in normal serum. Sugar was determined in serum which had been kept, for some time, in an incubator;

hence the figures are not exact. There was, perhaps, more sugar in tumor serum, but not enough cases were investigated to warrant a definite conclusion. Rolly and Oppermann found, in the few cases at their disposal, an increase of sugar in the plasma.¹ The two sera which gave contradictory results were, strangely enough, taken from animals in which inoculation occurred much earlier than in other cases. Whether these results were due to the resistance of the animal to tumor growth, or to the weakness of the tumor, must be left for further study to determine. For these two sera the tumors were very small and entirely encapsulated.

In summarizing the results the author feels justified in concluding that in the case of Rous's sarcoma, chemical analysis gives much more reliable results than other diagnostic methods. Several objections can, however, be put forward. The most important of these is that Rous's chicken sarcoma differs in many respects from other tumors; it is regarded by Rous himself as being of infective origin. In a few instances rat serum was used—of rats inoculated with Jensen's sarcoma. In each case 5–6 rats were bled and the bloods combined, so that the figures in the Tables represent good averages. Here, too, the differences were very much of the same order as for chicken sera. This matter awaits further study.

A second objection, no less important, is the fact that the same chemical differences may be found in other pathological conditions. The animals regarded as normal were brought up in town, and were kept for a long time in the laboratory. Everybody who has worked with fowls knows that, under such conditions, fowls do not develop normally. Incidentally, a few cases of avian tuberculosis were investigated among the non-tumor animals and normal figures were obtained.

We see, from the results in the accompanying Tables, that the size of the tumor does not seem to have an effect on the data, though possibly the length of the "inoculation period" played a part. This point will be studied in the near future. The chlorin content was found to be practically the same in tumor and normal sera, and therefore can be disregarded.

Although one is practically able to diagnose Rous's chicken sar-

¹ Rolly and Oppermann: *Biochem. Zeit.*, 1913, xlviii, p. 471.

coma from chemical data for the serum, it does not follow that analogous results can be obtained for human serum. Our study will be extended in this direction as soon as further clinical material is available. By using micro-methods, the amounts of blood to be taken can be very greatly diminished. Very interesting, also, will be the analysis of serum in pregnancy, where differences in other directions may be revealed.

The method is slightly inconvenient because normal serum must be taken as a control. From the figures obtained it is evident that, by working in pairs (one normal and one tumor serum), both results were relatively either high or low. This was due very likely to the fact that the birds were bled completely, and practically the whole serum was used for analysis.

TABLE I
Data pertaining to the alcoholic extraction method on fowls
(Blood from the throat: values per 100 gm. of serum)

Number	Alcoholic precipitate			Filtrate		Nature of the tumor	
	Quantity	N	P	N	P		
1 {	R 27.....	3.73	0.518	0.0116	0.0475	0.0141	Both sera hemolytic.
	N 4.....	5.37	0.764	0.0239	0.0475	0.0239	
2 {	R 12.....	4.56	0.656	0.0207	0.0408	0.0295	Hemolytic. Slightly hemolytic.
	N 5.....	3.62	0.511	0.0150	0.0353	0.0175	
3 {	R 79.....	4.00	0.566	0.0099	0.0303	0.0244	Slightly hemolytic.
	N 6.....	5.85	0.859	0.0093	0.0197	0.0156	
4 {	R 5.....	4.16	0.585	0.0245	0.0179	0.0422	Tumor small and firm.
	N 7.....	4.63	0.659	0.0104	0.0129	0.0154	
5 {	R 86.....	4.27	0.577	0.0144	0.0383	0.0258	Small, firm tumor.
	N 8.....	6.45	0.887	0.0124	0.0246	0.0134	
Average R...	4.14	0.588	0.0162	0.0349	0.0272		
N...	5.18	0.736	0.0142	0.0273	0.0171		

Experimental. 1. The method used was as follows: The blood was taken from pairs (one normal and one tumor animal), and was obtained by cutting the throat of the animal. Both bloods were left for the same length of time in an incubator. The separated serum was weighed and precipitated with 10 times its weight of alcohol. Both precipitates were left in the same desiccator and weighed, and an aliquot part taken for nitrogen and phosphorus estimations. The filtrate was diluted, with the alcoholic washings of the precipitate, to 250 cc.; 100 cc. were taken for each determi-

nation. In estimating small amounts of phosphorus, in a total volume of 50 c.c., by the method described in Hoppe-Seyler-Tierfelder, precipitation frequently failed to occur but could be obtained at greater dilutions.

These experiments (Table 1) show a larger quantity of alcoholic precipitate for normal animals, a corresponding increase in nitrogen, and a slight decrease in phosphorus. In the alcoholic filtrate for tumor animals, there were increases in nitrogen and phosphorus. The experiments were deficient, however, in the fact that, by cutting the throats, the contents of the crops possibly contaminated the blood. All the subsequent experiments on fowls were made with blood from the heart, taken by means of a canula, with the animal under anesthesia.

TABLE 2
Data pertaining to the alcoholic extraction method on rats
(Blood from the throat: values per 100 gm. of serum)

	Alcoholic precipitate			Filtrate	
	Quantity	N	P	N	P
Sarcoma.....	6.07	0.828	0.0104	0.064	0.0195
Normal.....	7.54	1.07	0.0240	0.051	0.0201

2. There were eight rats with Ehrlich's sarcoma and six normal ones in this series (Table 2). The method was that described above (1).

TABLE 3
Data pertaining to the alcoholic extraction method on fowls
(Blood from the heart: values per 100 gm. of serum)

	Alcoholic precipitate			Filtrate		Remarks	
	Quantity	N	P	N	P	Weight of tumor, grams	Inoculation period, days
1 { R 22..	3.39	Lost	0.0089	0.0347	0.0183	40	42
N 9..	3.94	0.547	0.0081	0.0193	0.0162	Trace of hemolysis	45
2 { R 59..	3.12	0.424	0.0104	0.0182	0.0160		
N 10..	4.82	0.690	0.0130	0.0211	0.0271	20	10
3 { R 65..	4.74	Lost	0.0107	0.0215	0.0145		
N 11..	3.71	0.535	0.0093	0.0184	0.0124	50	77
4 { R 15..	5.35	0.779	0.0219	0.0318	0.0304		
N 12..	4.47	0.616	0.0130	0.0293	0.0172	50	77
5 { R 5..	2.32	0.315	0.0063	0.0154	0.0113		
N 13..	2.91	0.407	0.0078	0.0219	0.0134	50	77
Aver. R..	3.78	0.505	0.0116	0.0243	0.0181		
N..	3.99	0.559	0.0102	0.0220	0.0172		

TABLE 4

Data pertaining to serum of fowls analyzed directly
(Blood from the heart; values per 100 gm. of serum)

No.	Serum							Remarks	
	N	P	S	Cl	NH ₂ -N	Sugar	Δ	Weight of tumor, grams	Inoculation period, days
1	R 15.....	0.602	0.019	0.070	0.255	—	—	5	27
	N 15.....	.784	.024	.066	.199	—	—		
2	R 53.....	.588	.020	—	—	—	—	120	22
	N 17.....	.875	.028	.061	.383	—	—	Avian tuberculosis	
3	R 3.....	.616	.028	.077	.286	—	—	50	15
	N 18.....	.889	.029	.071	.355	0.065	—		
4	R 49.....	.728	.021	.093	.340	—	—	10	17
	N 16.....	.871	.022	.077	.344	—	—		
5	R 134.....	.546	.025	.042	.394	.066	—	50	12
	N 19.....	.812	.028	.062	.397	.046	—	Lipemic	
6	R 132.....	.770	.026	.076	.284	.073	0.64	100	14
	N 20.....	.819	.030	.070	.390	.055	.65		
7	R 118.....	.749	.018	.090	.354	.031	.67	100	16
	N 21.....	.812	.028	.058	.354	.032	.64		
8	R 131.....	.580	.026	.054	.291	.032	.68	150	19
	N 22.....	.882	.028	.077	.425	.038	.74		
9	R 133.....	.609	.020	.050	.383	.019	.71	60	21
	N 23.....	.749	.020	.068	.397	.010	.74		
10	R 137.....	.714	.031	.059	.255	.014	.73	50	23
	N 24.....	.819	.037	.054	.256	.013	.75		
11	R 136.....	.798	.035	.058	.340	.020	.74	50	26
	N 25.....	.840	.037	.058	.369	.016	.81		
12	R 122.....	.672	.022	.077	.383	—	—	50	28
	N 26.....	1.155	.028	—	—	—	—	Avian tuberculosis	
13	R 1.....	.693	.029	.071	.390	.059	.63	3	20
	N 27.....	.833	.033	.069	.390	—	.66		
14	R 29.....	1.018	.035	.090	.397	—	.71	15	74
	N 28.....	.878	.028	.071	.411	—	.70	Firm, encapsulated, slowly growing	
15	R 4.....	.791	.039	.069	.390	—	.75	70	26
	N 29.....	1.008	.028	.088	.397	.154	.75		
16	R 66.....	.654	.037	.053	.383	—	.64	0.5	20
	N 30.....	.966	.029	.081	.397	—	.67		
17	R 8.....	.616	.039	.058	—	—	.67	100	33
	N 31.....	.714	.039	.066	.369	.016	.64		
18	R 120.....	.805	.035	.062	—	—	.70	1	65
	N 32.....	.668	.031	.066	—	—	.67	Slow growth, encap- sulated	
19	R 109.....	.770	.033	.059	.411	—	.68	10	25
	N 33.....	1.036	.046	.084	.372	—	.66	Metastases in the muscle	
20	R 48.....	.644	.032	.066	.397	.020	.64	50	17
	N 34.....	.763	.032	.063	.397	.014	.66		
21	R 123.....	.819	.057	.063	—	—	.65	15	12
	N 35.....	.868	.061	.079	—	—	.67	Hemolytic	
22	R 50.....	.500	.052	.057	—	—	.64	75	22
	N 36.....	.840	.072	.085	—	—	.66	Cysts in the liver	
	Aver. R.....	0.694	0.031	0.066	0.349	0.037	0.68		
	N.....	0.858	0.033	0.070	0.350	0.030	0.69		

3. In the third series (Table 3), where the inoculation period, and the size of the tumor, were noted, the average result was similar to that in the previous experiment (1); but we see that the size of the tumor had no effect, on the numerical values.

4. In the fourth series of experiments serum was always taken from pairs of animals, to insure reliable results. (Table 4.) We see, from the foregoing data, that nitrogen was *very much* higher, and phosphorus, sulfur, chlorine and molecular depression *slightly* higher, in normal serum. In the Rous sera, amino-nitrogen and sugar were slightly higher. Two tumor sera (Nos. 14 and 18) gave contradictory values. This may be due to the resistance of the body to tumor growth. In these two animals the tumor was older than in others but attained only a very small size, with a capsule separating it entirely from the rest of the muscle tissue.

TABLE 5

Data pertaining to serum analyzed directly.

(Serum of rats with Jensen's sarcoma: values per 100 gm. of serum.)

Serum	N	P	S	Sugar	Δ	Remarks
Sarcoma.....	1.050	0.075	0.356	0.070	0.61	Tumors 17 days old, hemolytic sera.
Normal.....	0.955	0.078	0.072	0.051	0.63	Hemolytic.

5. In the fifth experiment six rats with small tumors were bled by cutting the throats; the blood volumes were combined. Five normal rats were treated in the same way. (Table 5.)

We see, also, chemical changes in the same direction for rats. This result will be checked by further study.

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CONVENIENT METHODS FOR DEMONSTRATING
THE BIOCHEMICAL ACTIVITY OF MICRO-
ORGANISMS, WITH SPECIAL REFER-
ENCE TO THE PRODUCTION
AND ACTIVITY OF
ENZYMES

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(WITH PLATE I)

INTRODUCTION. A large amount of work has been done on the biochemistry of microorganisms and many reliable tests for demonstrating these activities have been evolved. Some of the tests are not well adapted for ocular demonstration to classes, however, because of their transitoriness or for other reasons. We have thought it worth while to describe in the following paragraphs a few methods, for making semi-permanent demonstrations of these activities, which seem adapted to the needs of class room or laboratory instruction. Some of the methods given here are adapted from those of other investigators; other methods are original with the present writers.

The methods are designed to show the presence and action of products of cellular activity upon appropriate substances incorporated in thin layers of agar in Petri dishes. Agar seems to serve very well for this purpose, since it is not difficult to prepare a clear solution, and most solutes diffuse readily through the gel which it forms. When solid zymolytes are used they are suspended in the agar. In order to procure uniform distribution of these solids through the medium, the plates are poured directly from the flask in which the medium is cooked and sterilized. By frequent shaking between pourings, settling of the solids is prevented. With this procedure, tubing of the medium is entirely unnecessary, if not detrimental to the best results. Stain reduction cannot be well

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shown by this Petri dish method, because the process of chemical reduction is usually counteracted by rapid oxidation of the products in contact with atmospheric oxygen.

For a number of the experiments a stock agar was prepared according to the following formula, filtered, and sterilized in the autoclave: *Distilled water*, 1000 c.c.; *magnesium sulfate*, 0.5 gm.; *di-potassium hydrogen phosphate*, 1.0 gm.; *potassium chlorid*, 0.5 gm.; *ferrous sulfate*, 0.01 gm.; *agar*, 20.0 gm. This stock medium is slightly acid in reaction. It presents no carbon-containing nutrient and consequently does not support microorganic growth. To this various zymolytes in the form of carbon containing compounds are added and inoculated with the organisms whose activities are to be tested.

TABLE I

Data pertaining to amylolytic tests on starch-agar.

Organism	Growth	Starch dissolution beneath centre	Halo produced
<i>Glomerella rufomaculans</i>	Good	Fair	None
<i>Sphaerostilbe coccophila</i>	"	Good	"
<i>Pseudopeziza ribis</i>	"	"	"
<i>Helminthosporium turcicum</i>	Poor	Weak	"
<i>Alternaria</i> sp.....	Good	Good	"
<i>Phyllosticta pirina</i>	"	"	Weak
<i>Septoria lycopersici</i>	None	—	—
<i>Aspergillus niger</i>	Good	Good	Excellent
<i>Oospora scabies</i>	"	"	"
<i>Streptothrix</i> sp.....	"	"	"
<i>Diplococcus</i> sp.....	Fair	Slight	None
<i>Micrococcus citricus</i>	Slight	"	"
<i>B. fluorescens liquifaciens</i>	"	"	"
<i>B. pyocyaneus</i>	"	"	"
<i>B. arogenes</i>	"	"	"
<i>B. denitrificans</i>	"	"	"
<i>Bact. tumefaciens</i>	"	"	"
<i>B. coli</i>	"	"	"
<i>Bact. lactis acidi</i>	None	—	—
<i>B. mycoides</i>	"	—	—
<i>B. prodigiosus</i>	"	—	—
<i>B. vulgaris</i>	"	—	—
<i>B. putidum</i>	"	—	—
<i>B. hayleibii</i>	"	—	—
<i>B. butyricus</i>	"	—	—
<i>B. campestris</i>	"	—	—

AMYLASE. The action of this enzyme may be conveniently demonstrated by cultivating organisms on starch-agar, made by adding to 500 c.c. of the melted stock agar, 10 gm. of corn starch

suspended in a little cold water. The medium is then sterilized in the autoclave and poured with frequent shaking directly from the flask into sterile Petri dishes. This gives a clear white substratum in which the starch is suspended. As soon as the agar has solidified, the centers of the dishes are inoculated with the organisms to be tested. The dishes are inverted and incubated for two to five days under bell jars to prevent loss of moisture. Typical results are shown in Table 1 and Plate 1, Fig. 1.

Some of the organisms produce extracellular amylase which diffuses from the colony, dissolves the starch suspended in the agar, and renders the space about the colony clear. Such an appearance is designated as a "halo." The presence of a halo, then, around the edge of a bacterial or fungous colony on starch-agar indicates the production, by that organism, of a readily diffusible extracellular amylase.

Some organisms, especially fungi, do not produce a halo on starch-agar, but grow well and dissolve the starch from the agar immediately in contact with them. In such a case the secretion of extracellular amylase is apparently weak or the amylase is not so diffusible as that produced by other organisms.

Among the organisms so far tested, *Streptothrix*, *Oospora scabies* and *Aspergillus niger* were found to be the most active producers of extracellular amylase. The first of these is able to produce abundant amylase and to dissolve large quantities of starch even in the presence of an abundance of sugar.

INULASE. Inulin is hydrolyzed by the enzyme inulase to reducing sugars, and as such may be utilized by microorganisms.

TABLE 2

Data pertaining to growth of fungi on inulin agar

Organism	Growth	Spore production
<i>Glomerella rufomaculans</i>	Fair	Fair
<i>Phyllosticta pirina</i>	Good	Good
<i>Coniothyrium pirinum</i>	Good	Poor
<i>Aspergillus niger</i>	Good	Good
<i>Streptothrix</i> sp.	Poor	Almost none

Inulin-agar. To 1000 c.c. of the stock agar, 0.5 percent of pure inulin was added, and Petri dish cultures made as in the case of the

starch-agar. Inulin is soluble in hot water and consequently dissolves during sterilization. Evidence of the ability of the organisms to dissolve inulin in this medium is afforded only by the amount of growth and spore production.

Only a few fungi have been tested, but some of them grew well upon this medium. See Table 2.

EMULSINS: glucoside-splitting enzymes. The glucosides are complex organic compounds capable of hydrolysis. Various end products, one of which is always a sugar, are the result. Since the glucosides are readily soluble, the agar containing them is transparent and the action of enzymes can be determined only indirectly. If the organisms can use the glucosides as sources of carbon, it is assumed that cleavage of the glucosides occurs in such consumption. The glucosides in a pure state are added to the stock agar to the amount of 1 percent, and plates poured and inoculated.

Esculin-agar. A bluish color pervades the agar made with esculin. In the event of the successful growth of the organism this blue color is reduced, sometimes throughout the plate. The successful growth of the organism is, however, a better indication of its ability to produce emulsin than is the color reduction.

Arbutin-agar. Arbutin, by hydrolytic cleavage, yields sugar and hydroquinone, which gives a brown color. The successful growth of the organism, and production of a brown stain on agar containing this chemical as the only source of carbon, are regarded as evidences of its ability to produce emulsin.

Amygdalin-agar. Success or failure to grow on this medium is our only indication of the ability or inability of an organism to produce emulsin. Typical data are given in Table 3.

LIPASE. Demonstration of the presence of lipase depends upon its power to split fats into glycerol and free fatty acids. The presence of the acids may then be shown by a convenient indicator.

Litmus-cream-agar. Fifty c.c. of 48 percent separator cream are diluted to 600 c.c. with distilled water and fractionally sterilized in an Arnold sterilizer. Twenty gm. of agar-agar are melted in 400 c.c. of water; the liquid is filtered, sterilized and added to the diluted cream while hot; and enough sterile litmus solution is poured into the fluid to impart a deep blue color. Plates are poured, and

TABLE 3

Data pertaining to tests for emulsin-production on amygdalin-agar

Organism	Growth
<i>Fusarium culmorum</i>	Excellent
<i>Alternaria</i> sp.	Fair
<i>Oospora scabies</i>	None
<i>Streptothrix</i> sp.	Good
<i>B. fluorescens liquifaciens</i>	Good
<i>B. coli</i>	Good
<i>Bact. lactis acidi</i>	Good
<i>B. denitrificans</i>	Good
<i>B. prodigiosus</i>	Fair
<i>Bact. tumefaciens</i>	Fair
<i>B. mycooides</i>	Slight
<i>B. campestris</i>	Slight
<i>M. citricus</i>	Slight

TABLE 4

Data pertaining to tests for lipase-production on litmus-cream-agar

Organism	Growth	Reddening of litmus*
<i>Sphærostilbe coccophila</i>	Good	None
<i>Helminthosporium turcicum</i>	Good	Good, diffusing
<i>Macrosporium</i> sp.	Good	Sharp
<i>Fusarium culmorum</i>	Good	Deep, diffusing
<i>Stysanus capitata</i>	Good	Slight
<i>Penicillium</i> sp.	Good	Sharp
<i>Oidium lactis</i>	Good	Good, diffusing
<i>Streptothrix</i> sp.	Good	Good, diffusing
<i>Azotobacter chroococcum</i>	None	—
<i>B. megatherium</i>	Good	Good, diffusing
<i>B. butyricus</i>	Good	Good, diffusing
<i>B. fluorescens liquifaciens</i>	Good	Deep
<i>B. mycooides</i>	Good	Good, diffusing
<i>B. proteus vulgaris</i>	Good	Sharp, brilliant
<i>B. pyocyaneus</i>	Good	Good, diffusing
<i>B. coli</i>	Good	Good, diffusing
<i>B. prodigiosus</i>	Good	Deep
Bacteria of ropy milk	Good	None, blue stain reduced
<i>M. citricus</i>	Good	Slight, diffusing
<i>Sarcina alba</i>	Good	Deep
<i>Diplococcus</i> sp.	Good	None

inoculated with various organisms. A medium containing approximately 2.5 percent of butter-fat and a very small amount of other

*“*Sharp*,” in the above table, indicates that the red area produced by the organism is sharply defined and in marked contrast to the surrounding blue. “*Diffusing*” indicates a gradual gradation from red through purple to blue, the acid apparently diffusing more rapidly than in the case of sharp contrast.

cream-constituents is thus prepared. Lipase excreted by the organism splits the butter-fat and the resulting free fatty acids turn the litmus red. In the case of several organisms the litmus is subsequently reduced to a colorless substance. Ropy-milk bacteria reduce the blue stain without first turning it red, and *Diplococcus* sp., although it grows fairly well, produces no change in the litmus. See Table 4.

All but one of the *fungi* tested cause production of acid from the cream-fat. *Macrosporium*, *Penicillium* and *Fusarium* show very marked lipolytic activity. Nearly all the *bacteria* tested are active in breaking up fat. Among the best are *B. proteus vulgaris*, *B. prodigiosus*, and *B. fluorescens liquifaciens*.

The fat in the cream-agar, prepared as above but without litmus, may be stained with alkannin. The lipolytic action of the bacteria growing on this medium brings about a reduction of the stain. Those organisms which show the strongest acid forming ability on the litmus-cream-agar give also the strongest reactions on alkannin-cream-agar.

A third series of plates of unstained cream-agar may be employed. The organisms most active on litmus and alkannin in the above tests are also most active in this series. No halo is produced however. Most of the *fungi* tested digest all of the fat in the agar immediately under the culture and leave it colorless.

Ethyl butyrate-litmus-agar, prepared as follows, may also be used: To 500 c.c. of the stock agar, 20 c.c. of saturated litmus solution are added. Enough sodium hydroxid sol. is introduced to give a slight alkaline reaction. Five c.c. of ethyl butyrate are then added, and the medium sterilized and poured into plates.

B. mycoides, *M. citricus* and *Oidium lactis* give a decided reddening of the agar as a result of acid production by hydrolysis of the ester. Nearly all of the organisms tested grow to some extent on this medium. It is however much less satisfactory than the litmus cream agar.

PROTEASES. The production of proteases is a familiar process, numerous examples of which may be seen daily in the laboratory. The peptonizing action of many organisms, when grown upon gelatin-media, is familiar to all.

The solvent action of enzymes is commonly apparent when microorganisms are grown upon Heyden Nährstoff-agar. This medium almost invariably contains particles of protein material which have passed through the cotton filters and give Petri dishes a slight turbidity. Colonies of organisms usually dissolve these particles through the action of proteases, the diffusing enzymes acting for some distance beyond the margin of the colony. More conspicuous examples of this power may be afforded by using some of the following methods.

TABLE 5

Data pertaining to tests for protease-production on fibrin-agar

Organism	Growth	Dissolution of fibrin
<i>Phyllosticta pirina</i>	Good	Rapid
<i>Helminthosporium turcicum</i>	Excellent	Very rapid
<i>Aspergillus</i> sp.	Good	Rapid
<i>Aspergillus niger</i>	Good	Slow
<i>B. pyocyaneus</i>	Very slight	Very slight
<i>Sarcina lutea</i>	Excellent	Good
<i>Diplococcus</i> sp.	None	—
<i>B. hartlebii</i>	Slight if any	Slight if any
<i>B. lactis arogenes</i>	Slight if any	Slight if any
<i>B. fluorescens liquifaciens</i>	Slight if any	Slight if any
<i>M. citricus</i>	Excellent	Good
<i>B. subtilis</i>	Excellent	Slight
<i>B. megatherium</i>	Excellent	Good
<i>Bact. lactis acidi</i>	Fair	Slight
<i>B. prodigiosus</i>	Good	Good
<i>B. campestris</i>	Good	Good
<i>B. mycoides</i>	Good	Good
<i>B. putidum</i>	Slight if any	Slight if any
<i>B. cyanogenus</i>	Slight if any	Slight if any
Bacteria of ropy milk	Fair	Fair
<i>B. butyricus</i>	Excellent	Excellent
<i>B. amylovorus</i>	None	—
<i>Microspira tyrogena</i>	Slight if any	Slight if any
<i>B. vulgaris</i>	Slight	Slight
<i>Bact. denitrificans</i>	Slight	Slight
<i>B. coli</i>	Slight	Slight
<i>Bact. tumefaciens</i>	Slight	Slight
<i>B. radiatum</i>	Fair	Slight
<i>Streptothrix</i> sp.	Excellent	Fair
<i>Oidium lactis</i>	Poor	Slight

Fibrin-agar. Some blood fibrin is pulverized in a mortar and added to the stock agar to the amount of 1 percent. Agglutination of the fibrin takes place and although it is almost impossible to get it evenly distributed through the medium, many fungi thrive on it satisfactorily and dissolve fibrin. Some bacteria show marked action on the fibrin but produce no distinct halo; others grow poorly

or not at all. Perhaps the failure of many of them to grow is not due to their inability to produce proteases but to the fact that the uneven distribution of the fibrin clumps makes it difficult for the organisms to get started. See Table 5 for typical data.

Protein-agar. A sample of commercial protein prepared from wheat is ground very fine in a mortar and added to the stock agar to the amount of 1 gm. per 100 c.c. Table 6 presents typical results.

TABLE 6
Data pertaining to tests for protease-production on protein-agar

Organism	Growth	Fruiting	Dissolution of protein particles
<i>Phyllosticta pirina</i>	Good	Good	Rapid
<i>Coniothyrium pirinum</i>	Good	Good	Rapid
<i>Helminthosporium turcicum</i>	Excellent	Excellent	Very rapid
<i>Aspergillus</i> sp. (green)	Poor	Poor	Slight
<i>Aspergillus</i> sp. (white)	Poor	Poor	Slight
<i>Endothia parasitica</i>	Fair	None	Rapid
<i>Aspergillus niger</i>	Fair	Good	Slight
<i>Glomerella rufomaculans</i>	None	—	—
<i>Rhizoctonia solani</i>	None	—	—
<i>Ascochyta colorata</i>	None	—	—
<i>B. prodigiosus</i>	None	—	—
<i>B. subtilis</i>	None	—	—
<i>B. pyocyaneus</i>	None	—	—
<i>B. amylovorus</i>	None	—	—
<i>B. campestris</i>	Slight	—	—
<i>Bact. tumefaciens</i>	Slight	—	—

EREPSIN. Erepsin hydrolyses the simpler proteins into amino acids, such as leucin and tyrosin. It is best demonstrated by its solvent action upon the simpler proteins.

Casein-agar. To the stock agar technical casein, in a finely divided condition, is added to the amount of 1 percent. This medium shows in an excellent manner the action of ereptic enzymes. Many of the organisms tested secrete erepsin in such quantity as to dissolve entirely all the casein in a wide band around the colonies, producing thereby a notable halo. A series of plates of casein agar was made in which the agar was rendered neutral to rosolic acid with ammonium hydroxid. All the organisms tested made a much weaker growth and produced much poorer halos on this series than on the slightly acid series. Erepsin is elaborated with greater rapidity and works best in the presence of a slight amount of acid.

The bacteria were grown only on the alkaline agar because they

could not survive on the acid medium. Typical data are given in Table 7.

TABLE 7
Data pertaining to tests for erepsin-production on casein-agar

Organism	Growth and fruiting	Dissolution of casein particles beneath culture	Halo produced
<i>Phyllosticta pirina</i>	Good	Good	None
<i>Aspergillus niger</i>	Good	Good	None
<i>Coniothyrium pirinum</i>	Good	Good	Good but narrow
<i>Glomerella rufomaculans</i>	Good	Good	Good but narrow
<i>B. arogenes</i>	—	Poor	
<i>B. pyocyaneus</i>	—	Good	
<i>B. campestris</i>	—	Good	
<i>B. amylovorus</i>	—	None	
<i>Bact. tumefaciens</i>	—	Fair	
<i>M. citricus</i>	—	Good	

TABLE 8
Data pertaining to tests for erepsin-production on skim-milk-agar

Organism	Growth	Dissolution of coagulum	Halo produced
<i>Sphaerostilbe coccophila</i>	Good	Slight	None
<i>Stysanus capitata</i>	Good	Good	Good
<i>Pseudopeziza ribis</i>	Good	Good	None
<i>Colletotrichum gloeosporoides</i>	Good	Good	None
<i>Glomerella gossypii</i>	Fair	Fair	Poor
<i>Helminthosporium turcicum</i>	Good	Fair	None
<i>Macrosporium solani</i>	Good	Good	None
<i>Alternaria</i> sp.	Good	Good	None
<i>Fusarium culmorum</i>	Good	Good	None
<i>Septoria lycopersici</i>	Poor	None	None
<i>Phyllosticta pirina</i>	Good	Good	None
<i>Penicillium pinophylli</i>	Good	Good	Fair
<i>Oidium lactis</i>	Good	—	Poor
<i>Actinomyces bovis</i>	Good	—	Poor
<i>Streptothrix</i> sp.	Good	—	Poor
<i>Azotobacter chroococcum</i>	Good	—	Poor
<i>B. campestris</i>	Good	—	None
<i>B. amylovorus</i>	None	—	None
<i>B. hartlebii</i>	Good	—	None
<i>B. fluorescens liquifaciens</i>	Excellent	—	Good
<i>B. butyricus</i>	Good	—	Good
<i>B. prodigiosus</i>	Excellent	—	Good
<i>B. mycoides</i>	Good	—	Good
<i>B. pyocyaneus</i>	Good	—	Good
<i>B. megatherium</i>	Excellent	—	Excellent
<i>B. putidum</i>	Poor	—	None
<i>Bact. lactis acidi</i>	Good	—	None
<i>B. arogenes</i>	Good	—	None
<i>M. citricus</i>	Good	—	Excellent
<i>M. candidans</i>	Poor	—	None
<i>Sarcina alba</i>	Excellent	—	None
<i>Microspira tyrogena</i>	Poor	—	None
<i>Diplococcus</i>	Excellent	—	None
<i>Urea coccus</i>	Good	—	None

Skim-milk-agar. Some separator skim milk is diluted with an equal volume of water and 20 gm. of agar added per liter. This mixture is heated for half an hour in an autoclave to coagulate the casein. The medium is then boiled in an Arnold sterilizer for 4 hrs., when the casein is in a very finely divided condition. Plates are prepared in the usual way. See Table 8.

This medium is most excellent for the demonstration of erepsin secretion as shown in Plate 1, Figs. 2, 3, and 4. The most satisfactory organisms to use for the demonstration of ereptic activities on skim-milk-agar are: *B. megatherium*, *M. citricus*, *B. butyricus*, *B. fluorescens liquifaciens*, *B. prodigiosus* and *B. pyocyanus* in the order named. Among those tested which showed least activities are: *B. campestris*, *M. candidans*, *B. hartlebii*, *Bact. lactis acidi*, and *B. lactis aerogenes*.

Peptone-agar is also very useful in the demonstration of erepsin. It is prepared by adding Witte peptone (1 percent) to the stock agar. Good halos are produced by many organisms.

TRYPSIN. One of the most convenient methods of demonstrating the action of trypsin, produced by microorganisms, depends upon the use of egg-albumen-agar. Many microorganisms grow readily upon egg-albumen-agar and digest the coagulum.

TABLE 9

Data pertaining to the action of certain organisms on egg-albumen-agar

Organism	Growth	Digestion
<i>B. campestris</i>	Good	Good
<i>Sarcina lutea</i>	Good	Very good
<i>B. pyocyanus</i>	Great	Very good
<i>Oidium lactis</i>	Great	Slight
<i>B. subtilis</i>	Good	Slight
<i>B. mycoides</i>	Good	None
<i>B. prodigiosus</i>	Good	Good
<i>B. megatherium</i>	Good	Good
<i>Streptothrix</i> sp.	Good	Slight
<i>Bact. lactis acidi</i>	Fair	Slight
<i>Bact. tumefaciens</i>	Small	None
<i>B. amylovorus</i>	Small	None
<i>B. vulgaris</i>	Slight	None
<i>B. lactis aerogenes</i>	Slight	None
<i>B. butyricus</i>	Small	Slight
<i>B. putidum</i>	Small	Slight

Egg-albumen-agar is prepared by taking the "whites" of three eggs and beating them in 75 c.c. of water with a Dover egg-beater. This material is then added to 500 c.c. of melted stock agar, shaken,

and cooked for a half hour in an autoclave. The cooking process breaks the coagulated albumen into small particles and gives a cloudy medium. This agar is then poured into Petri dishes and, when solid, inoculated with the desired organisms. As the colonies develop they digest the coagulum and a halo of clear agar surrounds the colony (Plate 1, Fig. 5). The results of tests of the tryptic activity of certain organisms on egg-albumen-agar are given in Table 9.

AMIDASE. Amidase is an enzyme capable of attacking amino compounds and forming ammonia along with other cleavage products. Upon this liberation of ammonia is based the following test.

Asparagin-rosolic-acid-agar. To the stock agar is added 0.5 percent of asparagin and 5 c.c. of 2 percent rosolic acid per liter. The rosolic acid, which is yellow with acid, gives this medium a slight yellow tint. The liberation of ammonia from the asparagin renders the medium alkaline, and the rosolic acid takes on a brilliant red color. This is a beautiful reaction and a highly satisfactory test for the production of amidase by lower organisms. See Table 10.

TABLE 10

Data pertaining to tests for amidase-production on asparagin-rosolic-acid-agar

Organism	Growth	Reaction
<i>B. fluorescens liquifaciens</i> ...	Good	Deep brilliant red, diffusing
<i>B. putidum</i>	Very good	Deep red
<i>B. arogenes</i>	Good	Red, little diffused
<i>B. prodigiosus</i>	Fair	Slight red, little diffused
<i>Bact. lactis acidi</i>	Very good	Deep red, widely diffused
<i>B. denitrificans</i>	Very good	Deep red, widely diffused
<i>Bact. tumefaciens</i>	Very good	Deep red, widely diffused
<i>B. pyocyaneus</i>	Very good	Deep red, widely diffused
<i>B. coli</i>	Very good	Deep red, widely diffused
<i>Actinomyces bovis</i>	Very good	Deep red, widely diffused
<i>B. hartlebii</i>	None	—
<i>B. butyricus</i>	Slight or none	No change
<i>B. megatherium</i>	Slight	No change
<i>B. radiatum</i>	Slight	No change
<i>B. campestris</i>	Slight	No change
<i>B. amylovorus</i>	Slight	No change
<i>B. vulgaris</i>	Slight	No change
<i>B. subtilis</i>	Slight	No change
<i>B. mycoides</i>	Slight	No change
<i>M. citricus</i>	Slight	No change
<i>Sarcina alba</i>	Slight	No change
<i>Sarcina lutca</i>	Slight	No change
<i>Oospora scabies</i>	None	No change
<i>Streptothrix</i> sp.	None	No change
<i>Pseudopeziza ribis</i>	Good	Slight red
<i>Glomerella rufomaculans</i> ...	Very poor	No change
<i>Helminthosporium turcicum</i> ..	Poor	Very deep red, not diffused

Cultures showing active production of ammonia from asparagin and reddening of the agar were kept for 8 to 10 days. Some of them, at the expiration of this time, showed a yellowing of the medium about the centre of the culture. This is notably true of *B. putidum* and *B. pyocyaneus*. The indications are that subsequent to the production of ammonia an acid is produced, due, no doubt, to bacterial action on the cleavage products of the asparagin.

CYTASE. The presence and action of cellulose-dissolving enzyme has been for a long time demonstrable microscopically. Recently, however, Kellerman and McBeth² have described a convenient Petri-dish method for demonstrating the cellulose-dissolving action of bacteria and fungi.

The medium used in our experiments was prepared according to Kellerman's method, as follows: Exactly 400 c.c. of ammonium hydroxide (sp. g. 0.90) were diluted to 600 c.c. and an excess of copper carbonate added. After standing all night, the excess of copper carbonate settled to the bottom and the supernatant solution was siphoned off. Seven and one half gm. of dry filter paper were added and the product shaken up at intervals. In a few minutes solution of the cellulose was complete. The solution was diluted to 5 liters and a 1 : 5 solution of hydrochloric acid added, a few c.c. at a time, until the cellulose settled to the bottom. Water was added to make 10 liters, the cellulose allowed to settle and the supernatant liquid decanted. The cellulose was then washed with repeated changes of water containing a little hydrochloric acid, until the blue color of the solution disappeared. It was then washed with distilled water until a silver-nitrate test showed the washings to be free from chlorid.

The cellulose was suspended in 500 c.c. of water containing magnesium sulphate, 0.25 gm.; potassium biphosphate, 0.5 gm.; potassium chlorid, 0.25 gm.; ferrous sulfate, trace; sodium nitrate, 1.0 gm.; agar agar, 10.0 gm. The mixture was autoclaved and poured into plates.

² Kellerman and McBeth: The fermentation of cellulose, *Centralbl. f. Bakt., Abt. 2*, 34: 485 (1912). Kellerman: The excretion of cytase by *Penicillium pinophilum*, *Circ.* 118, *Bur. Pl. Ind., U. S. Dept. Agr.*, 1913. McBeth and Scales: The destruction of cellulose by bacteria and filamentous fungi, *Bull. 226, Bur. Pl. Ind., U. S. Dept. Agr.*, 1913.

It is not always easy to obtain organisms which produce cytase abundantly. Kellerman has described, in the papers cited, some species of aerobic bacteria and fungi which he found to be active. Mixed cultures of these organisms may be obtained by selective cultivation on the following medium: filter paper, in strips 2.0 gm.; ammonium chloride, 0.1 gm.; potassium biphosphate, 0.05 gm.; calcium carbonate, 2.0 gm.; water, 100.0 c.c.

This medium is placed in large Erlenmeyer flasks to form a layer about one centimeter in depth. The flasks are inoculated with fresh horse-manure, slimy mud from a pond, or with sewage from a septic tank in active operation. After a month's incubation transfers are made to new flasks containing the same medium. In this way the cellulose-destroying organisms are selected. The strips of filter paper first become brown and then perforated with holes, and a brown color is usually imparted to the solution. Transfers may then be made to cellulose-agar in tubes or Petri dishes.

Söhngen³ has recently described a method for demonstrating the cytolytic powers of bacterial colonies by the use of manganese compounds. A sheet of filter paper is dipped in a manganese sulfate sol. and then in sol. of potassium permanganate. The resulting manganic oxid is held in the paper and gives it a brown color. This sheet is dried and sterilized in a Petri dish, then moistened with a nutrient solution and inoculated with cellulose-destroying bacteria. The acids formed in course of the destruction of cellulose combine with the manganic oxid to form light colored salts, which present a conspicuous contrast on the dark background.

FORMATION OF LACTIC ACID AND OTHER ACIDS. It has been known for some time that the addition of calcium carbonate to agar gives a method for demonstrating the action of acids produced by bacteria. Frequently failures have resulted from the difficulty of adding just the proper amount of calcium carbonate. The method which we have employed obviates this difficulty.

Beef-peptone agar containing 1 percent of lactose is made and put into test tubes as usual. Before plugging the tubes a small quantity of calcium carbonate (0.5 to 2.0 gm.) is added to each tube. The

³ Söhngen: Umwandlungen von Manganverbindungen unter dem Einfluss mikrobiologischer Prozesse, *Centralbl. f. Bakt., 2te Abt.*, 40: 545 (1914).

agar is then given triple sterilization in an Arnold sterilizer. Most of the carbonate settles to the bottom of the tubes, but a small amount of finely divided material remains in suspension in the agar and renders it distinctly turbid. After the final sterilization, the supernatant, turbid, agar is poured into sterile Petri dishes, care being taken to prevent carbonate in the bottom of the test tube from passing into the dishes.

The turbid agar was inoculated with *Bacterium lactis acidi* and placed in the incubator for three days. At the end of that time the colonies showed distinct halos due to the solvent action of acids upon the calcium carbonate. (Plate I, Fig. 6.)

Another convenient method of demonstrating the presence of acids is the familiar one of adding litmus solution to medium containing lactose. The method is so well known to all bacteriologists that it is unnecessary to repeat it here.

Another indicator which is useful in this connection is rosolic acid. A few drops of 0.5 percent solution are added to tubes of sterile lactose-agar before melting and pouring it into Petri dishes. The results are shown in Table II.

TABLE II

Data pertaining to effects of organisms upon the color of rosolic acid

Organism	After 13 days	After 28 days
<i>Glomercella rufomaculans</i>	Deep pink	Beginning to fade
<i>Fusarium lycopersici</i>	Unchanged	Colorless
<i>Sterigmatocystis niger</i>	Slight change	Deep pink
<i>Bacillus subtilis</i>	No growth	
<i>B. pyocyaneus</i>	No growth	

The alkaline conditions observed were probably due to the formation of ammonia from protein constituents.

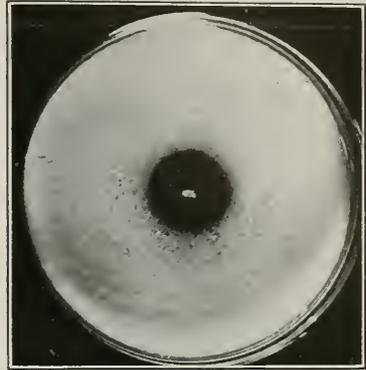
Numerous other applications of these various methods will suggest themselves to students of these problems. When only qualitative results are required, it is believed these methods will be found highly satisfactory. An added advantage lies in the ease with which permanent records may be made for the student's note book. The Petri dishes may be set on photographic or blue-print paper and exposed to light. The paper, after development, may be inserted in the note book as a part of the record.

EXPLANATION OF PLATE 1

- FIG. 1. Solvent action of *Streptothrix* on starch-agar.
- FIG. 2. Colony of *Macrosporium solani* on milk-agar.
- FIG. 3. Large colony of *B. megatherium* on milk-agar.
- FIG. 4. Action of *M. citricus* on milk-agar.
- FIG. 5. Solvent action of *Sarcina lutea* on egg-albumen-agar. This print was made by placing the Petri dish on the photographic paper and illuminating it for the proper time.
- FIG. 6. Solvent action of *Bact. lactis acidi* on calcium carbonate suspended in the agar.



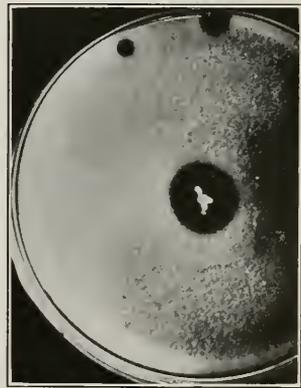
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6

CRABILL AND REED: CONVENIENT METHODS FOR DEMONSTRATING THE BIOCHEMICAL ACTIVITY OF MICROORGANISMS

REACTION OF RABBITS TO INTRAVENOUS INJECTIONS OF MOULD SPORES¹

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(WITH PLATE 2)

The work outlined in this paper was undertaken in connection with experiments having as their object a determination of the chemical differences which may exist between the two sexual races in the fungous group commonly known as the mucors. In these forms the majority of the species are dioecious, having separate male and female races, which may be propagated independently by means of vegetative spores.

As is well known the repeated injection of red blood corpuscles and of certain other cells is capable of producing in the blood of rabbits cytolytic antibodies that will dissolve these cells when they are subsequently mixed with the serum of the treated animal. It was thought that a similar cytolysin might be developed for mould spores, and that the action might be found to be sexually specific.

Although agglutinins apparently are formed, no cytolysins for fungus spores could be induced by intravenous injections. Despite the largely negative character of the results obtained, they seem to be sufficiently controlled to show positively that rabbits are incapable of producing cytolysins for the spores of the mucor tested. The fact that the cell wall is highly resistant throughout the fungi renders it extremely improbable that cytolysins can be developed for spores of any other fungus form.

Preliminary tests showed that spores of *Cunninghamella echinulata*, when injected intravenously, kill a rabbit within a week's time (four instances). Postmortem examination demonstrated the pres-

¹ The major part of the work embodied in this paper was carried out at the Station for Experimental Evolution of the Carnegie Institution of Washington, Cold Spring Harbor, New York.

See Proceedings of the Columbia University Biochemical Association, Dec. 4, 1914; *BIOCHEM. BULL.*, 1915, iv, p. 212.

ence of germinated spores in the lungs. This mould is a tropical form growing readily at temperatures above 31° C., and its growth in the rabbits may have caused death by mechanically interfering with the functions of the organs infected.

Most species of the mucors will not grow at temperatures over 30° C. Among the forms that grow only at relatively low temperatures, "Mucor V"—a form similar to *M. hiemalis*, if not identical with this species—gives an especially strong sexual reaction. Its spores are relatively small (about $8 \times 3.5 \mu$) and can offer little interference to the circulation. No strong toxins, moreover, are developed by this mould such as have been found in the allied form *Rhizopus nigricans* (1, 2). Altogether, the species seemed especially favorable and has been used in the present investigation.

The mold was grown, generally on agar, in shallow pie-tins protected with paper covers. Water was poured over the mature culture and filtered through linen which allowed the spores to pass while keeping back fragments of the aerial mycelium which it was feared might block the capillaries. The spore-water was centrifuged and the resulting compacted mass of spores was mixed with 0.9 percent salt solution and used at once for the injections. Centrifuged spores were dried in a vacuum desiccator and in a few instances were used later when fresh spores were not available. The injections were all made in an ear vein, the usual aseptic precautions being observed. The dose varied from 3 to 4 c.c. The spore-water was always very dark with spores and, although counts were not made each time, the individual injections can be considered to average about 500,000,000 spores.

Rabbit No. 5, beginning April 2, 1913, received at intervals of about 4 days, 28 preliminary injections of the spores of the ♂ race (3) of "Mucor V." Rabbit No. 55, beginning April 17, similarly received 27 preliminary injections of the ♀ spores of the same species. On August 13, five days after the last injection, rabbits Nos. 5 and 55 received approximately 800,000,000 spores, respectively, of the ♂ and ♀ races; and at the same time two control rabbits, Nos. 6 and 66, previously untreated, were similarly injected with like doses of the ♂ and ♀ spores, respectively. This made the 29th injection for rabbit No. 5, and the 28th for No. 55. The four

TABLE I

Figures specify number of viable spores recovered from blood of treated and control rabbits at intervals indicated

Hours after final injection		½	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7½	8½	9½	10½	12½	14½	16½	18½	21	24	27	30	36	43	50	73	91	Totals	
Animals injected on Aug. 13 with about 800,000,000 ♂ spores	Rabbit No. 5	17	24	12	79	79	141	145	152	215	92	104	212	276	414	179	149	100	81	0	9		4	8	8	5	1	1	0	0	0	0	2597
	Previously treated with ♂ spores																																
	Rabbit No. 6	12	121	35	97	139	121	30	99	18	48	44	18	38	15*	56	32	46	11	0	0	2	0	1	1	2	0	0	0	0	0	986	
Animals injected on Aug. 13 with about 800,000,000 ♀ spores	Rabbit No. 55	32	11	33	80	34	67	44	42	36	34	15	37	83	32	51	9	36	7	10	5	3	1	0	0	0	0	0	0	0	0	0	702
	Previously treated with ♀ spores																																
	Rabbit No. 66	48	98	16	76	138	170	79	54	80	27	29	31	8	51	28	12	5	15	6	2	1	4	2	1	0	0	0	0	0	0	981	
Total number of spores from Nos. 5 and 6, injected with ♂ spores.		29	145	47	176	218	262	175	251	233	140	148	230	314	429	235	181	146	92	0	9	6	8	9	6	3	1	0	0	0	0	3493	
		80	109	49	156	172	237	123	96	116	61	44	68	91	83	79	21	41	22	16	7	4	5	2	1	0	0	0	0	0	0	1683	
		49	35	45	159	113	208	189	194	251	126	119	249	359	446	230	158	136	88	10	14	7	9	8	5	1	1	0	0	0	0	3209	
		60	219	51	173	277	291	109	153	98	75	73	49	46	66	84	44	51	26	6	2	3	4	3	2	2	0	0	0	0	0	0	1967
Total number of spores from Nos. 6 and 66, controls.		109	254	96	332	399	499	298	347	349	201	192	298	405	512	314	202	187	114	16	16	10	13	11	7	3	1	0	0	0	0	5176	

* About half the number of spores in this tube were lost by the breaking of the tube.

rabbits were all females of about the same age. At the time of the final injections No. 5 weighed 2130 gm.; No. 6, 2160 gm.; No. 55, 1900 gm.; No. 66, 1730 gm.

The number of spores were estimated by counts with a hemacytometer. Loops of blood were taken from these rabbits at half hourly, hourly, and eventually at less frequent intervals. The blood, taken from a slight prick in an ear vein, was at once mixed with sterile water in small vials, and as soon as possible made into separation cultures in Lindner roll-tubes (4). The last injection was made in the left ear and the blood to be tested was always taken from the right ear.

TABLE 2

Figures indicate number of viable spores in loop taken from tubes of serum or salt solution at intervals indicated

		Time of incubation in hours					Totals
		24	34	45	56	80	
About 12,000,000 ♂ spores added with 0.9 per cent. salt solution to 0.5 c.c. of serum	Rabbit No. 5						
	Previously injected with ♂ spores	315	13	29	2	0	359
	Rabbit No. 55						
	Previously injected with ♀ spores	89	111	32	7	0	239
	Rabbit No. 80						
	Not injected (control)	205	88.	31	3	0	327
About 12,000,000 ♀ spores added with 0.9 per cent. salt solution to 0.5 c.c. of serum	Rabbit No. 5						
	Previously injected with ♂ spores	105	13	1	0	0	119
	Rabbit No. 55						
	Previously injected with ♀ spores	107	60	12	0	0	179
	Rabbit No. 80						
	Not injected (control)	92	58	0	1	0	151
♂ spores in 0.9 per cent. salt solution without serum		750	675	325	26	0	1776
♀ spores in 0.9 per cent. salt solution without serum		500	462	42	14	0	1018

Table 1 shows the number of viable spores in the loops taken, as indicated by the number of colonies developing in these roll-tube separation cultures. The quantities of blood taken up in the loops unavoidably varied, and thus no doubt, influenced considerably the number of spores found at each test. The number of spores injected into the rabbits could not be exactly the same and they may have been for some time unequally distributed in the blood. Such experimental errors may account for a considerable variation in the number of spores found in the individual loops but can hardly explain the relatively large total count for rabbit No. 5. An individual peculiarity of this animal may be responsible for the larger

number of spores recovered from its blood. After about 10 hours, viable spores gradually disappear from the blood stream and are no longer found after 43 hours. It is of significance that they disappear from the blood of the rabbits which had been previously treated with spores, no sooner than from the controls. No cytolytins, therefore, or other antibodies capable of destroying the spores have been developed in response to previous injections.

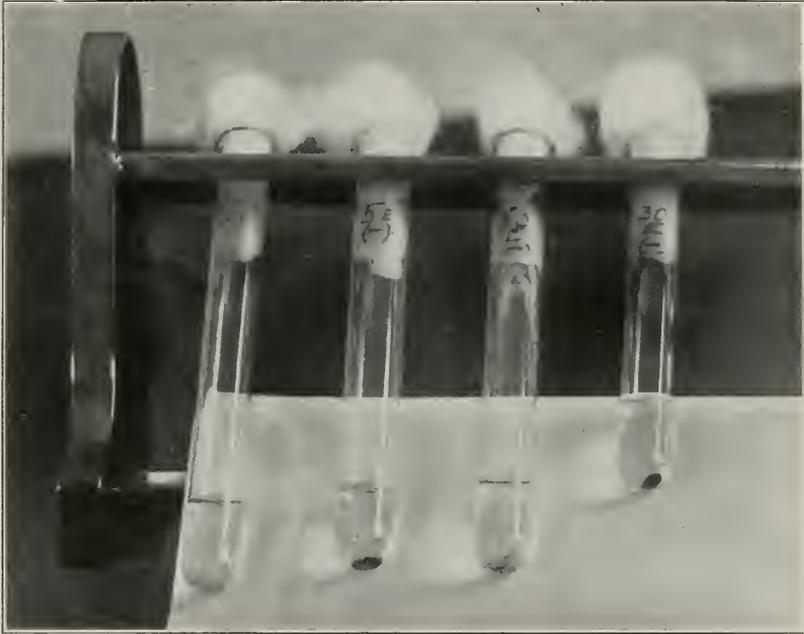
Each of the treated rabbits received an enormous number of spores during the period of injections and must have disposed of them in some way—how, has not been determined. Postmortems on animals 5 and 55 failed to show abnormal conditions of the viscera. Further, the lungs, liver, spleen and kidneys were preserved and later examined microscopically but failed to give evidence of any local accumulation of spores. The spores have hyaline walls and may not greatly differ from blood corpuscles in size or in appearance, when either of these are for any reason distorted. It seemed desirable, therefore, to postpone search after the fate of the injected spores until a form might be used with walls that could be easily identified.

A series of experiments was performed *in vitro* with the sera of the treated rabbits. Animals 5 and 55 were bled Aug. 22, nine days after their last injection. On Aug. 24, the action of their sera and that of a control ♀ rabbit No. 80 was tested on ♂ and ♀ spores. The number of spores was roughly determined with a hemacytometer. One cc. of the spore mixture containing in the neighborhood of 12,000,000 spores was added to 0.5 cc. of the different sera and, together with controls in salt solution alone, the tubes were placed in the incubating oven. It is not impossible that rather more ♂ than ♀ spores were added to each tube and this fact would explain the uniformly high counts for all tests of ♂ spores in this series. The results are shown in Table 2 and merely confirm the facts brought out in Table 1—*i. e.*, that the sera of the treated rabbits developed no antibodies for the destruction of spores.

Although no cytolytins were developed, there was found evidence of the formation of agglutinins in the blood of the treated animals. On Aug. 26, about 6,000,000 ♂ and ♀ spores, respectively, were added in 0.9 percent salt solution to various dilutions of the sera of the same rabbits (Nos. 5, 55 and 80), and controls were

made with ♂ and ♀ spores alone in salt solution. The volume in each tube was 1.5 cc. They were incubated at 37° C. The spores in the salt solution, in settling, formed a uniform layer on the bottom of the tube. In the control serum (No. 80), at dilutions greater than 1:600, the spores also settled uniformly. In dilutions up to 1:1,200, the sera of Nos. 5 and 55 caused a distinct clumping of both ♂ and ♀ spores, producing an irregular roughening of the edges of the spore mass at the bottom of the tubes. A slight but distinct reaction could be observed in the sera of Nos. 5 and 55 with ♀ spores, and in serum No. 5 with ♂ spores at a dilution of 1:2,400. A similar clumping of spores was found in the control serum (No. 80) but only at the tested dilutions of 1:30, 1:150, and 1:300. At dilutions of 1:600, 1:1,200, and 1:2,400 no clumping of spores was observed in the control serum. The reaction seems comparable to the agglutination produced with bacteria. The tests failed to show any specific sexual reaction such as had been suggested in precipitin tests with the "press-saft" from the mycelium of this same mold (5).

It was not possible to carry on the work with the sera further at this time. They were inactivated at 56° C. for one half hour on Aug. 26, and later taken to Storrs, Conn., where they were preserved in an icebox. Pressure of other work prevented a repetition of the tests until Feb. 15, 1914. Despite the age of the sera the results were in general confirmatory of the previous tests. Fresh serum from another rabbit (No. 30) was used as a control. The spore mixture was estimated to contain somewhat over twice as many spores as were previously employed. Their greater number may in a measure account for the fact that in the higher dilutions of the control serum the spores, instead of forming a uniform layer on the bottom of the test tubes as had been the case in the control serum six months before, now settled together into a dense dot. Plate 2 shows ♂ spores in tubes of serum at a 1:320 dilution, together with spores in salt solution alone. The tubes were slightly slanted after the spores were added and the slant reversed to show the character of the settled spores in the photograph. The roughening of the edges of the spore masses in sera Nos. 5 and 55 is a characteristic appearance. The spores in No. 55 were slightly disturbed in handling.



BLAKESLEE AND GORTNER: REACTION OF RABBITS TO INTRA-
VENOUS INJECTIONS OF MOULD SPORES

Five sixths natural size. Photograph showing male spores of *Mucor V* in tubes of the following fluids, reading from left to right: 0.9 per cent. salt solution, serum of rabbit No. 5, serum of rabbit No. 55, serum of control rabbit No. 30. The different sera were all at a 1:320 dilution.

The sera of Nos. 5 and 55 were tested with spores of two other molds,—*Absidia glauca* and a species of the *Mucor racemosus* type. There seemed to be a slight agglutination with both species. It is possible that the rabbits tested had received so many spore injections that their sera had largely lost their specificity and so were reactive to spores of any species of this mold group. The results do not seem to warrant many definite conclusions in regard to the agglutination reaction but suggest the need of further investigation.

The results outlined in the preceding pages show that repeated intravenous injections into rabbits of fungus spores fail to cause the development in their blood of cytolytic substances capable of dissolving such spores, but apparently stimulate the formation of antibodies that cause them to agglutinate.

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STUDIES ON THE PHYSICO-CHEMICAL PROPERTIES OF VEGETABLE SAPS

3. A comparison of the physico-chemical constants of the juices expressed from the wall with those from the included carpelary whorl in proliferous fruits of *Passiflora gracilis*

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(WITH PLATE 3)

I. **Introduction.** In this paper we present a limited portion of the data which we have gathered concerning the physico-chemical properties of the expressed juices of morphologically differentiated fruits of *Passiflora gracilis*.

Our purpose in carrying out this work has been to ascertain whether there may not be chemical differences underlying, or at least associated with, morphological differences in plant organs. The fruits of *Passiflora gracilis* furnish excellent material for such a study. One of us (Harris, 1906) has described some of the more common abnormalities in these fruits. Later (Gortner and Harris, 1913) we attempted on a small scale to ascertain whether there is any consistent chemical difference between normal and teratological fruits. The results of this investigation led to work with improved methods of analysis during the summer of 1913, when some 800 individual samples (400 teratological and an equal number of normal controls) of fruits were examined. All of these data have not as yet been assembled; results from another season's cultures will be secured before conclusions are drawn concerning certain of the relationships.

This paper is limited to a comparison of the properties of the juices expressed from the wall of the abnormal fruit with those from the mass of abnormal tissue which it contains.

The proliferous fruit of *P. gracilis* is characterized in the simplest cases by the production, from the base of the fruit, of a stalked whorl or series of whorls of incompletely closed carpels. In such cases the proliferation must be regarded as morphologically a continuation of the axis, whose activity generally closes with the formation of the carpels constituting the normal ovary. The included or abnormal mass, as we shall sometimes designate this accessory carpellary whorl, is generally large, green and turgid, but it may be small and shrivelled. It may become so large as to rupture the normal ovary wall. The variation in structural details is very great and will form the subject of a forthcoming memoir by one of us.

The fruits which have furnished the materials for this paper have been selected with great care as to morphological type. They comprise exclusively those which are trimerous (the normal condition, *i. e.*, with six external sutures and three placentae) in the organization of their wall, and have a large living basal proliferation with an external whorl of four carpels. The diagrams (Plate 3) make clear the type of fruit dealt with. Numerous detail drawings will be published when the morphological problems are taken up.

2. **Material.** The materials upon which the constants were determined were drawn from five cultures of *P. gracilis* grown under slightly varying conditions on the grounds of the Station for Experimental Evolution in the summer of 1913. All the plants were typical and (relatively) normally grown.¹ These five series do not include all of the fruits taken into consideration in our work, but since the other experiments were made under highly abnormal conditions of growth for special purposes, it seems quite legitimate to leave them out of account here.

Three of the cultures represent a strain which has been under cultivation at the Station since the summer of 1908, when the seed was secured from an American dealer. The second lot of seed was a commercial sample bought from Haage und Schmidt, of Erfurt. The series considered² are:

¹ *P. gracilis* is an exotic; while it grows magnificently in our latitude when started under glass, it is probably not quite legitimate to speak of normal conditions of growth.

² All seeds were germinated under glass and transferred to 8 cm. pots before planting into the garden.

CULTURE A. American seeds. The young plants were transferred to large pots³ of garden soil, which were plunged in the garden early in May. Collections were made from 24 plants.

CULTURE B. This was carried out in precisely the same manner as *Culture A*, with the exception that 175 gm. of bone meal was thoroughly mixed with the soil of each pot. Collections were made from 25 plants.

CULTURE C. This and the following culture was made from the Haage und Schmidt seed, which was germinated considerably later than the lot that gave rise to the preceding cultures. The young plants were transferred directly from 8 cm. pots into the garden. They were divided into two lots, the first of which (*Culture D*) was placed in a garden somewhat separated from that in which Cultures A to C were grown. Altogether there were 43 plants.

CULTURE D. The plants (30) were identical with those of *Culture C* but grown in the garden separate from that in which Cultures A-C were grown. (The gardens were about 200 yards apart.)

CULTURE E. A number (53) of plants from the same sowing as experiments A and B were potted up in 15 cm. pots, in soil which was considerably better than that in the garden. After a few days in the greenhouse to allow the plants to become established, these pots were plunged in the garden adjoining Cultures A and B.⁴

The distribution of the collections from which the abnormal fruits were drawn is shown in Table 1.

The entries in this table show that in exp. C and D the materials were gathered from well matured vines late in the fall, whereas in the other three series the collections were made at intervals throughout the season. Thus, the entire materials of *Culture C* were taken for analysis from Sept. 29 to Oct. 3, inclusive. All the

³ These had an inside diameter of about 29 cm. at the top and 20 cm. at the bottom, with a depth of 30 cm.

⁴ The plan was to give these plants a limited root space, but, through a misunderstanding on the part of the gardener, the holes in the bottom of the pots were not sufficiently stopped and the soil was drawn up over the tops of the pots, so that roots passed through the bottom and others over the top of the pots. As a result these plants made a more vigorous growth than those in the larger pots.

fruits of exp. D were brought in for analysis between 3 P.M. Oct. 6 and 3 P.M. Oct. 7. The large series E furnished samples for the chemical laboratory from Aug. 19 to Oct. 16. The two short series were grown in large pots sunk in the ground, and furnished abnormal fruits of the kind here considered from Sept. 10 to Oct. 6 (exp. A) and from Aug. 15 to Oct. 4 (exp. B). Thus, three of these collections were made over a wide period, during which the plants were subjected to great variations in meteorological conditions. Furthermore, these repeated collections from the same individuals must be composed of fruits representing the plant in very different physiological stages. Sufficient proof of this assertion is to be found in the demonstration that the proportion of teratological fruits becomes smaller in successive collections (Harris and Gortner, 1914).

This table by no means includes all of the fruits produced by the plants. Others remained on the vines after the discontinuation of collections for chemical purposes because of the lateness of the season. Furthermore, large series of fruits were dissected and recorded for morphological purposes but omitted from the chemical samples because not sufficiently mature.

TABLE I

Data pertaining to distribution of the collections from which the abnormal fruits were drawn

Date	A	B	C	D	E	Totals
August 15-16.....	—	442	—	—	—	442
August 19-22.....	275	301	—	—	404	980
August 25.....	135	—	—	—	—	135
August 29-Sept. 4.....	—	2438	—	—	768	3206
September 10-11.....	3640	—	—	—	—	3640
September 12-20.....	—	—	—	—	16131	16131
September 23-25.....	—	11820	—	—	2927	14747
September 26-27.....	6519	—	—	—	—	6519
September 29-Oct. 3 ..	—	—	9570	—	—	9570
October 4-7.....	3096	4096	—	8369	—	15561
October 14-16.....	—	—	—	—	23643	23643
Totals.....	13665	19097	9570	8369	43873	94574

With regard to this last point, the greatest care was taken to secure fruits at as nearly the same stage of development as possible. In the gathering of the fruits only those with dried calyces

were intentionally taken. The fruits were scrutinized again with regard to this character and to maturity of seeds as they were dissected. In general, only fruits whose seeds were on the average more than half covered by the aril were used. All the fruits which showed any indication of the whitening of the wall which precedes the development of the red color, assumed in ripening, were discarded, as were also those in which the arils had assumed very much color.

Before leaving the question of materials, it may not be out of place to indicate the relative weights of the fruit wall (freed from seeds) and of the abnormal carpellary mass. The averages for the individuals samples are given in Tables 2-6.

The average of the mean weights for the three series of observations which are large enough to make it worth while to compute the probable errors are:

Experiment	Wall	Proliferation	Difference
C	1.848 ± 0.038	1.229 ± 0.019	-0.619 ± 0.043
D	1.740 ± 0.044	1.207 ± 0.038	-0.533 ± 0.058
E	1.583 ± 0.030	1.005 ± 0.041	-0.578 ± 0.051

Thus, the mean weight of the included mass is only two thirds of that of the fruit wall. In only a single sample does the weight of the proliferation exceed that of the ovary wall.

3. Experimental methods. A. COLLECTION OF MATERIAL. In any study of cell sap it is essential that the juices be obtained in a condition as nearly as possible identical with that in which they occur in the vacuoles of the protoplasm. In gathering the fruits, every reasonable precaution consistent with the magnitude of the task which had to be accomplished was taken to (a) secure fruits in a condition free from external moisture or contamination; (b) to protect them from loss of moisture by evaporation; (c) to preserve the wall of the ovary and the included mass, after dissection, in such a way as to incur as little change as possible in the conditions of the tissues; and, (d) above all, to avoid bringing about any artificial differences between the compared samples.

To attain these ends the fruits were collected with great care and dissected as soon and as rapidly as possible. The proliferous

fruits were placed, as soon as found, in a container with moistened bibulous paper and closed by ground glass or a rubber sealed cover. Additional proliferous fruits were added to the container until a sample sufficiently large for the expression of sap was obtained. The abnormal mass was not removed from the fruits until the sample was prepared for chemical analysis. The very exacting task of dissecting over 100,000 fruits would have been an almost impossible undertaking but for the untiring zeal of Miss Margaret Gavin, Miss Lily Gavin, Miss Edna K. Lockwood and Mr. Charles W. Crane, for whose assistance we desire to express our sincere thanks.

B. EXTRACTION OF THE SAP. The included mass was separated from the ovary wall. After weighing, the samples were packed separately in thick walled test tubes, which were tightly closed with rubber stoppers and, as an extra precaution, capped with oiled paper fastened around the neck of the test tube by a tightly drawn rubber band. The tubes were then plunged in a mixture of finely chopped ice and salt at a temperature of -17° , or lower (care being taken to keep the mouths of the test tubes above the freezing mixture). The freezing box was placed in a larger ice box to maintain the lowest possible temperature⁵ and allowed to remain at least 10 hours to insure the complete freezing of the tissues (See Gortner and Harris, 1914). This method of freezing the tissues is less expensive than that by liquid air (Dixon and Atkins, 1913) and, for large samples, is much more convenient and apparently quite as effective.

The contents were removed (after being carefully thawed) with great care, to prevent any contamination, folded in a small square of heavy muslin cloth (which had been boiled through three changes of distilled water and dried at 110° in the absence of dust), and the juice expressed by means of a small, heavily tinned, "beef-juice" press. The liquid was centrifuged at high speed to remove suspended solids and the physico-chemical constants determined on the clear sap.

C. DETERMINATION OF THE SPECIFIC ELECTRICAL CONDUCTIVITY. The electrical conductance, κ , at 30° , was determined in the

⁵ After ten hours the temperature of the ice and salt solutions was still as low as -8° to -10° .

usual manner,⁶ using a Freas conductivity cell⁷ having a cell constant of 0.4119, obtained by taking the specific conductance of 0.1 *N* KCl as 0.01412 at 30°.

D. DETERMINATION OF THE DEPRESSION OF THE FREEZING POINT. The depression of the freezing point was carried out by the well known Beckmann method, using the modifications we have suggested (Gortner and Harris, 1914), which make for more rapid work. The freezing point of the sap from the proliferous mass was determined immediately after that of the sap from the corresponding ovary walls. All freezing points were corrected for the concentration caused by the separation of ice due to undercooling.

$$\Delta = \Delta' - 0.0125 u \Delta'$$

where Δ' is the observed depression, u is the degrees of undercooling and Δ is the corrected depression of the freezing point.

E. DETERMINATION OF THE SPECIFIC GRAVITY. The specific gravity at 20° of the plant sap was obtained by weighing in a small pycnometer holding 5.6830 gm. of water at 20°. The maximum error in the specific gravity determination is not greater than ± 0.0002 .

F. DETERMINATION OF THE CONCENTRATION OF THE SOLUTES IN THE SAP. The concentration of the sap $\frac{\text{Wt. solutes}}{\text{Wt. solvent}}$ was determined by evaporating to dryness, at the temperature of a water oven, exactly 10 c.c. of the sap in a small weighing bottle. The weighing bottles were then placed in vacuum desiccators over conc. sulfuric acid and the desiccators exhausted to a pressure of less than 30 mm. Hg, and allowed to stand for at least 10 hours. They were then weighed. Drying at 105°–110° gives a smaller value for solids, but we believe that this is not caused by a further

⁶ Cf. Abderhalden: *Handb. d. Biochem. Arbeitsmethoden*, I, 485–498, or any good manual of physical chemistry.

⁷ This type of cell is very well suited for rapid work. The electrodes are held rigidly in place by small glass rods so that the cell may be shaken to remove traces of moisture. The cell was frequently tested with 0.1 *N* KCl, but during a period of nearly a year (during which time we have made some 1500 determinations of electrical conductivity), the cell constant has not changed. Cleaning the cell with chromic-sulfuric acid does not alter the cell constant.

loss of water but by the evaporation of some volatile constituent, or else by the decomposition of organic compounds. There seems to be some decomposition even when the drying is done in a water oven. Perhaps a more ideal method would be to dry *in vacuo* over sulfuric acid without the aid of heat, but owing to the large number of samples with which we had to deal such a procedure was not feasible.

$$\text{Concentration} = \frac{s}{10d - s}$$

where s is the solids in 10 c.c. and d is the specific gravity at 20°.

G. DETERMINATION OF THE MEAN MOLECULAR WEIGHT OF THE SOLUTES. The average molecular weight of the dissolved substances is given by

$$M = \text{Conc.} \times \frac{1860}{\Delta},$$

the calculation of which is facilitated by tables published elsewhere in this Journal: 3, p. 259-263, 1914.

4. Discussion of data. The individual constants are given in the accompanying series of tables (2-21). Numbers in the left hand columns are the laboratory numbers of the samples.

A. SPECIFIC GRAVITY AND CONCENTRATION. (Data, Tables 2-6 and 17-21.) These are the simplest possible measures of the properties of the sap of the two kinds of tissues.

It would be surprising if there were not distinct differences between the means of the specific gravities of samples taken from separate cultures and at different times. The data for mean specific gravities in the appended summary are for the three larger series:

Experiment	Wall	Proliferation
C	1.020168 ± 0.000154	1.017831 ± 0.000156
D	1.019225 ± 0.000115	1.018125 ± 0.000119
E	1.020636 ± 0.000231	1.019500 ± 0.000247

TABLES 2-6

Data pertaining to mean weight of parts of fruit and specific gravity of sap
Table 2, Series A

Sample number	Mean weight			Specific gravity of sap		
	Wall	Proliferation	Difference	Wall	Proliferation	Difference
76-86	1.424	0.640	-0.784	1.0215	1.0195	-0.0020
90	1.445	0.724	-0.721	1.0202	1.0193	-0.0009
207	1.666	0.858	-0.808	1.0186	1.0168	-0.0018
292	1.526	1.073	-0.453	1.0191	1.0180	-0.0011

Table 3, Series B

1	—	—	—	1.0194	1.0187	-0.0007
2+5	—	—	—	1.0202	1.0213	+0.0011
34	1.233	1.073	-0.160	1.0230	1.0223	-0.0007
35	1.133	0.950	-0.183	1.0234	1.0229	-0.0005
64	1.300	0.882	-0.418	1.0207	1.0198	-0.0009
189	1.582	0.885	-0.727	1.0204	1.0179	-0.0025
286	1.463	1.145	-0.318	1.0199	1.0193	-0.0006

Table 4, Series C

210	1.676	1.212	-0.464	1.0221	1.0204	-0.0017
211	1.970	1.280	-0.690	1.0201	1.0182	-0.0019
213	1.710	1.235	-0.475	1.0195	1.0178	-0.0017
214	1.780	1.330	-0.450	1.0200	1.0172	-0.0028
221	1.654	1.122	-0.532	1.0220	1.0193	-0.0027
228	—	—	—	1.0215	1.0183	-0.0032
237	1.507	1.030	-0.477	1.0193	1.0170	-0.0023
238	2.160	1.233	-0.927	1.0199	1.0173	-0.0026
245	1.915	1.165	-0.750	1.0191	1.0173	-0.0018
246	2.077	1.209	-0.868	1.0198	1.0170	-0.0028
247	2.181	1.272	-0.909	1.0197	1.0171	-0.0026
248	1.956	1.217	-0.739	1.0190	1.0169	-0.0021
260	1.827	1.438	-0.389	1.0201	1.0177	-0.0024
261	1.855	1.305	-0.550	1.0198	1.0179	-0.0019
262	1.406	1.031	-0.375	1.0204	1.0173	-0.0031
269	2.041	1.352	-0.689	1.0204	1.0186	-0.0018

These values furnish the data for the accompanying comparisons for the wall and the abnormal mass.

Comparison	Difference	d/E_d	Comparison	Difference	d/E_d
C and D	0.000943 ± 0.000192	4.91	C and D	0.000294 ± 0.000196	1.50
C and E	0.000468 ± 0.000277	1.68	C and E	0.001669 ± 0.000292	5.71
D and E	0.001411 ± 0.000258	5.46	D and E	0.001375 ± 0.000274	5.02

There can be no reasonable question of the significance of four of these differences. The factors involved in producing them are far too complicated to justify any attempt to explain them at present. Possibly they are in part due to differences in the strains

TABLES 2-6 (continued)

Data pertaining to mean weight of parts of fruit and specific gravity of sap
Table 5, Series D

Sample number	Mean weight			Specific gravity of sap		
	Wall	Prolification	Difference	Wall	Prolification	Difference
304	1.768	1.468	-0.300	1.0197	1.0191	-0.0006
305	1.990	1.585	-0.405	1.0196	1.0182	-0.0014
310	1.405	1.100	-0.305	1.0187	1.0175	-0.0012
311	1.455	1.090	-0.365	1.0183	1.0171	-0.0012
315	1.917	1.352	-0.565	1.0196	1.0182	-0.0014
316	1.710	1.035	-0.675	1.0195	1.0190	-0.0005
321	1.760	1.005	-0.755	1.0188	1.0176	-0.0012
324	2.010	1.255	-0.755	1.0185	1.0177	-0.0008
328	1.861	1.076	-0.785	1.0185	1.0176	-0.0009
329	1.352	0.910	-0.442	1.0201	1.0188	-0.0013
330	1.620	1.285	-0.335	1.0199	1.0182	-0.0017
347	2.031	1.318	-0.713	1.0195	1.0185	-0.0010

Table 6, Series E

21	1.411	1.283	-0.128	1.0200	1.0195	-0.0005
24	1.205	0.925	-0.280	1.0214	1.0202	-0.0012
28	1.200	0.805	-0.395	1.0230	1.0221	-0.0009
40	1.253	0.773	-0.480	1.0187	—	—
41	1.500	1.070	-0.430	1.0183	1.0177	-0.0006
102	1.550	0.683	-0.867	1.0208	1.0188	-0.0020
103	1.550	0.683	-0.867	1.0210	1.0191	-0.0019
118	1.576	0.881	-0.695	1.0194	1.0186	-0.0008
119	1.576	0.881	-0.695	1.0203	1.0188	-0.0015
131	1.512	0.752	-0.760	1.0226	1.0210	-0.0016
141	1.650	0.606	-0.954	1.0219	1.0205	-0.0014
146	1.772	0.813	-0.959	1.0186	1.0174	-0.0012
147	1.656	1.036	-0.620	1.0191	1.0170	-0.0021
153	1.756	0.712	-1.044	1.0203	1.0173	-0.0030
364	1.835	1.195	-0.640	1.0190	1.0180	-0.0010
365	1.760	1.450	-0.310	1.0211	1.0206	-0.0005
377	1.795	1.282	-0.513	1.0202	1.0202	±0.
392	1.773	1.226	-0.547	1.0218	1.0211	-0.0007
407	1.462	1.537	+0.075	1.0194	1.0198	+0.0004
408	1.866	1.411	-0.455	1.0239	1.0228	-0.0011

of plants employed; probably they are in part due to differences in cultural and meteorological conditions.

The mean concentrations in the three large series are shown in the accompanying summary.

Experiment	Wall	Prolification
C	0.04065 ± 0.00043	0.03591 ± 0.00049
D	0.03943 ± 0.00029	0.03705 ± 0.00028
E	0.04224 ± 0.00061	0.04009 ± 0.00069

These data give differences for the constants for the juice from the wall and from the mass:

Wall			Juice from Prolifcation		
Comparison	Difference	d/E_d	Comparison	Difference	d/E_d
C and D	0.00122 ± 0.00052	2.35	C and D	0.00114 ± 0.00056	2.04
C and E	0.00159 ± 0.00075	2.12	C and E	0.00418 ± 0.00085	4.92
D and E	0.00281 ± 0.00068	4.13	D and E	0.00304 ± 0.00074	4.12

The results are in substantial agreement with the findings for specific gravity.

We have been considerably surprised at the narrow limits of variation in specific gravity. The observed ranges are:

Experiment	Wall	Prolifcation
A	1.0186 — 1.0215 = 0.0029	1.0168 — 1.0195 = 0.0027
B	1.0194 — 1.0234 = 0.0040	1.0179 — 1.0229 = 0.0050
C	1.0190 — 1.0221 = 0.0031	1.0169 — 1.0204 = 0.0035
D	1.0183 — 1.0201 = 0.0018	1.0171 — 1.0191 = 0.0020
E	1.0183 — 1.0239 = 0.0056	1.0170 — 1.0228 = 0.0058

If one expresses the variation, in specific gravity, in the more scientific terms of the standard deviation,⁸ the results are, for the three large series:

Experiment	Wall	Prolifcation	Difference
C	0.000910 ± 0.000108	0.000926 ± 0.000110	+0.000016 ± 0.000141
D	0.000594 ± 0.000081	0.000612 ± 0.000084	+0.000018 ± 0.000100
E	0.001497 ± 0.000163	0.001600 ± 0.000175	+0.000103 ± 0.000244

The entries in this summary show, as do the ranges given above, that the variation in specific gravity within a particular series is very low indeed.

For both wall and proliferation the variability in specific gravity, as measured in terms of the standard deviation, is higher in exp. E than in either of the others. Thus, numerically the differences between exp. C and E are:

For wall, 0.000587 ± 0.000195 , $d/E_d = 2.99$;

For proliferation, 0.000674 ± 0.000207 , $d/E_d = 3.26$;

while that between the same constants, for D and E, are:

⁸ For the method of calculating the standard deviation, and the probable errors of means of series of observations as given in this paper, the reader must consult texts on higher statistics.

For wall, $0.000903 \pm 0.000182, d/E_a = 4.96;$

For proliferation, $0.000988 \pm 0.000194, d/E_a = 5.09;$

There can be no reasonable question of the trustworthiness of the difference. Compare the same constants for exp. C and D:

For wall, $0.000316 \pm 0.000135, d/E_a = 2.34;$

For proliferation, $0.000314 \pm 0.000138, d/E_a = 2.27;$

Note that the absolute differences are much lower and that the probable errors are relatively higher.

The ranges of variation observed in the concentration of solutes are:

Experiment	Wall	Proliferation
A	$0.0377 - 0.0429 = 0.0053$	$0.0342 - 0.0379 = 0.0037$
B	$0.0400 - 0.0482 = 0.0083$	$0.0357 - 0.0462 = 0.0105$
C	$0.0376 - 0.0463 = 0.0087$	$0.0329 - 0.0437 = 0.0107$
D	$0.0374 - 0.0412 = 0.0038$	$0.0347 - 0.0391 = 0.0044$
E	$0.0358 - 0.0490 = 0.0132$	$0.0333 - 0.0482 = 0.0149$

Or, expressed in terms of the square root of mean square deviation from the mean (standard deviation), the fluctuation is

Experiment	Wall	Proliferation
C	0.00240 ± 0.00031	0.00270 ± 0.00034
D	0.00150 ± 0.00021	0.00142 ± 0.00020
E	0.00383 ± 0.00043	0.00432 ± 0.00049

Series E shows a greater variability in concentration, just as was found to be true for specific gravity.

The explanation of these results probably lies in the wider range of external environmental conditions and internal physiological states prevailing during the collection of certain of the series as explained in a preceding section of this paper.

When one turns to a comparison of wall and proliferation he finds that the entries in the individual tables show that there are 55 cases in which the specific gravity of the sap of the proliferation is lower than that of the wall, to 2 cases in which it is higher. In one instance they are identical. The means for the three series in which the number of observations is large enough to justify the calculation of a probable error are:

Experiment	Wall	Proliferation	Difference
C	1.02017 ± 0.00015	1.01783 ± 0.00016	-0.00234 ± 0.00022
D	1.01923 ± 0.00012	1.01813 ± 0.00012	-0.00110 ± 0.00017
E	1.02064 ± 0.00023	1.01950 ± 0.00025	-0.00114 ± 0.00033

TABLES 7-11

Data pertaining to specific electrical conductivity, and to ratio of electrical conductivity to depression of the freezing point

Table 7, Series A

Sample Number	Specific Conductivity			κ/Δ		
	Wall	Proliferation	Difference	Wall	Proliferation	Difference
76+86	0.013272	0.009984	-0.003288	0.001574	0.001256	-0.000318
90	0.013166	0.010025	-0.003141	0.001569	0.001241	-0.000328
207	0.013404	0.008950	-0.004454	0.002219	0.001682	-0.000537
292	0.013350	0.009039	-0.004311	0.002225	0.001586	-0.000639

Table 8, Series B

I	0.011224	0.009413	-0.001811	0.001819	0.001551	-0.000268
2+5	0.011972	0.010180	-0.001792	0.001979	0.001549	-0.000430
13	0.011592	0.009105	-0.002487	—	—	—
34	0.013287	0.010691	-0.002596	0.002193	0.001764	-0.000429
35	0.012410	0.010222	-0.002188	0.002021	0.001630	-0.000391
64	0.013774	0.010518	-0.003256	0.001892	0.001496	-0.000396
189	0.015692	0.009285	-0.006407	0.002444	0.001661	-0.000783
286	0.012878	0.008631	-0.004247	0.002132	0.001460	-0.000672

Table 9, Series C

210	0.015297	0.010174	-0.005123	0.002195	0.001563	-0.000632
211	0.015236	0.009942	-0.005294	0.002355	0.001697	-0.000658
213	0.015545	0.009780	-0.005765	0.002519	0.001765	-0.000754
214	0.015988	0.009660	-0.006328	0.002483	0.001744	-0.000739
221	0.015114	0.009820	-0.005294	0.002184	0.001610	-0.000574
228	0.015482	0.009820	-0.005662	—	—	—
237	0.014347	0.009153	-0.005194	—	—	—
238	0.016248	0.015054	-0.001194	0.002492	0.002693	+0.000201
245	0.014637	0.009462	-0.005175	0.002361	0.001705	-0.000656
246	0.015988	0.009740	-0.006248	0.002486	0.001787	-0.000699
247	0.015670	0.009501	-0.006169	0.002411	0.001700	-0.000711
248	0.014405	0.009619	-0.004786	0.002389	0.001829	-0.000560
260	0.015733	0.009861	-0.005872	0.002466	0.001745	-0.000721
261	0.015420	0.009740	-0.005680	0.002436	0.001736	-0.000700
262	0.016379	0.010148	-0.006231	0.002524	0.001852	-0.000672
269	0.016248	0.010400	-0.005848	0.002429	0.001781	-0.000648

The differences may be regarded, in all cases, as statistically trustworthy in comparison with their probable errors.

If we express in terms of percentages, some care in selecting the base is necessary. In these solutions the unity term of the specific gravity is a constant which is enormously large as compared with the variability in the constants for the saps of the two tissues under investigation. If the difference in the specific gravities be divided by the actual specific gravity of either of the two samples, a ratio not at all comparable with the others to be considered later will be secured. We have, therefore, taken the ratio of the *difference* $\times 100$ to the variable term, *i. e.*, *d-I*, of the specific gravity,

TABLES 7-11 (continued)

Data pertaining to specific electrical conductivity, and to ratio of electrical conductivity to depression of the freezing point

Table 10, Series D

Sample Number	Specific Conductivity			κ/Δ		
	Wall	Prolifcation	Difference	Wall	Prolifcation	Difference
304	0.014233	0.009501	-0.004732	0.002337	0.001616	-0.000721
305	0.014233	0.008815	-0.005418	0.002307	0.001538	-0.000769
310	0.012673	0.008167	-0.004506	0.002126	0.001485	-0.000641
311	0.012422	0.007790	-0.004632	0.002074	0.001391	-0.000683
315	0.013138	0.008415	-0.004723	0.002112	0.001503	-0.000609
316	0.012372	0.008486	-0.006114	0.002012	0.001448	-0.000564
321	0.012078	0.007790	-0.004288	0.001961	0.001432	-0.000529
324	0.012623	0.008631	-0.005992	0.002083	0.001550	-0.000533
328	0.011508	0.007393	-0.004115	0.002019	0.001344	-0.000675
329	0.010400	0.007589	-0.002811	0.001745	0.001315	-0.000430
330	0.012175	0.007689	-0.004486	0.001908	0.001344	-0.000564
347	0.011792	0.007623	-0.004169	0.001863	0.001299	-0.000564

Table 11, Series E

21	0.014744	0.011133	-0.003611	0.002551	0.002028	-0.000523
24	0.014685	0.010822	-0.003863	0.002431	0.001905	-0.000526
28	0.014803	0.011499	-0.003304	0.002317	0.001923	-0.000394
40	0.014336	0.010180	-0.004156	0.002602	—	—
41	0.013556	0.010475	-0.003081	0.002487	0.002014	-0.000473
102	0.014178	0.010164	-0.004014	0.002126	0.001686	-0.000440
103	0.014235	0.010246	-0.003989	0.002160	0.001725	-0.000435
118	0.013085	0.009721	-0.003364	0.002097	0.001631	-0.000466
119	0.013190	0.009487	-0.003703	0.002032	0.001592	-0.000440
131	0.014061	0.009766	-0.004295	0.001997	0.001466	-0.000531
141	0.012821	0.009458	-0.003363	0.001842	0.001460	-0.000382
146	0.014402	0.009196	-0.005206	0.002353	0.001684	-0.000669
147	0.014576	0.009534	-0.005042	0.002325	0.001766	-0.000559
153	0.013083	0.008084	-0.004999	0.002087	—	—
364	0.013297	0.008926	-0.004371	0.002242	0.001611	-0.000631
365	0.013675	0.008704	-0.004971	0.002110	0.001397	-0.000713
377	0.013244	0.008778	-0.004466	0.002161	0.001488	-0.000673
392	0.011602	0.008063	-0.003539	0.001799	0.001305	-0.000494
407	0.013191	0.009115	-0.004076	0.002236	0.001582	-0.000654
408	0.011885	0.008595	-0.003290	0.001693	0.001296	-0.000397

d , of the wall. Expressed in this way the specific gravity of the sap of the proliferation is from 5.50 to 11.50 percent lower than that of the wall.

Turning now to concentration, we note that in only one instance does the concentration of the solutes in the sap of the proliferation equal or exceed that of the ovary wall. The averages are:

Experiment	Wall	Prolifcation	Difference
C	0.04065 ±0.00043	0.03591 ±0.00049	-0.00474 ±0.00065
D	0.03943 ±0.00029	0.03705 ±0.00028	-0.00237 ±0.00040
E	0.04224 ±0.00061	0.04009 ±0.00069	-0.00215 ±0.00091

B. SPECIFIC ELECTRICAL CONDUCTIVITY. (Data, Tables 7-11.) The electrical conductance of plant saps is in a large measure due to the presence of inorganic salts. Organic acids probably play a very minor rôle because of their low ionization constants.

The mean values and the probable errors of mean values for the conductivities of the saps of the two parts of the proliferous fruit are, for the three series in which the number of determinations are numerous enough to make it worth while to calculate probable errors, given in the appended summary:

Experiment	Wall	Prolification	Difference
C	0.015483 ±0.000103	0.010117 ±0.000220	-0.005366 ±0.000244
D	0.012470 ±0.000199	0.008157 ±0.000116	-0.004313 ±0.000224
E	0.013632 ±0.000133	0.009597 ±0.000140	-0.004035 ±0.000200

Confining our attention for the moment to the mean values, we note (*a*) that they are of about the order of 0.1 N KCl; and (*b*) that they differ significantly from series to series. Thus, the difference between Series C and Series D is, for the wall, 0.003013 ± 0.000224; for the prolification, 0.001053 ± 0.000332. The difference between Culture D and Culture E is, for the wall, 0.001162 ± 0.000217; for the prolification, 0.001440 ± 0.000181. These differences are several times as large as their probable errors.

The range of variation in observed conductance from sample to sample is indicated below:

Experiment	Wall	Prolification
A	0.013350 — 0.013404 = 0.000054	0.008950 — 0.010025 = 0.001075
B	0.011592 — 0.015692 = 0.004100	0.008631 — 0.010691 = 0.002060
C	0.014347 — 0.016379 = 0.002032	0.009153 — 0.015054 = 0.005901
D	0.010400 — 0.014233 = 0.003833	0.007393 — 0.009501 = 0.002108
E	0.011602 — 0.014803 = 0.003201	0.008063 — 0.011499 = 0.003436

Expressing the results in terms of the standard deviation we find:

Experiment	Wall	Prolification
C	0.000610 ± 0.000073	0.001305 ± 0.000156
D	0.001026 ± 0.000141	0.000599 ± 0.000082
E	0.000884 ± 0.000094	0.000931 ± 0.000099

Any regularity of the kind noted for the constants hitherto discussed cannot be detected. One can neither assert (*a*) that the tissues of the wall show more (or less) variability in the concentration of electrolytes than do those of the included mass, nor (*b*) that the series collected during a limited period of time show less variability than those collected over a considerable period.

A glance at the tables of data shows a very consistent difference between the electrical conductance of the sap of the ovary wall and that of the abnormal mass. In no instance is the concentration of salts in the proliferation as high as the concentration in the fruit wall. The absolute difference between the electrical conductivity of the sap of the wall and that of the proliferation is remarkably constant. The differences between the averages are given with their probable errors in the above summary. The differences are about 20 times as large as their probable errors. Thus there is no possible question of the trustworthiness of the difference between the conductance of the sap expressed from the ovary wall and that from the included mass. The conductance of the sap from the mass is 34.7 percent, 34.6 percent, and 29.6 percent lower than that from the wall. Note that these are higher percentage differences than those obtained for the specific gravities. To this point we shall return later.

C. DEPRESSION OF THE FREEZING POINT AND OSMOTIC PRESSURE. (Data, Tables 12-16.) The osmotic pressure is due to both the organic and inorganic solutes. It has been calculated from the corrected depression of the freezing point by means of the formula

$$P = 12.060 \Delta - 0.021 \Delta^2.$$

The work was facilitated by the tables which we have published elsewhere (Harris and Gortner, 1914). For purposes of the present discussion, the values of Δ and P may be taken as equivalent. Since most workers are more accustomed to think in terms of atmospheres-pressure, we shall tabulate values of P rather than Δ .

TABLES 12-16

Data pertaining to depression of the freezing point and osmotic pressure

Table 12, Series A

Sample number	Depression of the freezing point			Osmotic pressure in atmospheres		
	Wall	Prolifcation	Difference	Wall	Prolifcation	Difference
76-86	0.843°	0.795°	-0.048°	10.15	9.574	-0.576
90	0.839°	0.808°	-0.031°	10.10	9.730	-0.370
207	0.604°	0.532°	-0.072°	7.276	6.410	-0.866
292	0.600°	0.570°	-0.030°	7.228	6.867	-0.361

Table 13, Series B

I	0.617°	0.607°	-0.010°	7.433	7.312	-0.121
2+5	0.605°	0.657°	+0.052°	7.288	7.914	+0.626
34	0.606°	0.606°	±0	7.300	7.300	±0
35	0.614°	0.627°	+0.013°	7.396	7.553	+0.157
64	0.728°	0.703°	-0.025°	8.768	8.467	-0.301
189	0.642°	0.559°	-0.083°	7.733	6.735	-0.998
286	0.604°	0.591°	-0.013°	7.276	7.120	-0.156

Table 14, Series C

210	0.697°	0.651°	-0.046°	8.395	7.842	-0.553
211	0.647°	0.586°	-0.061°	7.794	7.059	-0.735
213	0.617°	0.554°	-0.063°	7.433	6.674	-0.759
214	0.644°	0.554°	-0.090°	7.757	6.674	-1.083
221	0.692°	0.610°	-0.082°	8.335	7.348	-0.987
238	0.652°	0.559°	-0.093°	7.854	6.735	-1.119
245	0.620°	0.555°	-0.065°	7.469	6.686	-0.783
246	0.643°	0.545°	-0.098°	7.745	6.566	-1.179
247	0.650°	0.559°	-0.091°	7.830	6.735	-1.095
248	0.603°	0.526°	-0.077°	7.264	6.337	-0.927
260	0.638°	0.565°	-0.073°	7.685	6.807	-0.878
261	0.633°	0.561°	-0.072°	7.625	6.759	-0.866
262	0.649°	0.548°	-0.101°	7.818	6.602	-1.216
269	0.669°	0.584°	-0.085°	8.058	7.035	-1.023

The means for the three larger series are, in terms of osmotic pressure, the following:

Experiment	Wall	Prolifcation	Difference
C	7.790 ± 0.055	6.847 ± 0.065	-0.943 ± 0.085
D	7.345 ± 0.041	6.831 ± 0.035	-0.514 ± 0.054
E	7.626 ± 0.081	7.126 ± 0.078	-0.499 ± 0.112

Comparing, we find the differences shown below:

Comparison	Wall	Prolifcation
C and D	0.445 ± 0.069	0.016 ± 0.074
C and E	0.164 ± 0.098	0.279 ± 0.101
D and E	0.281 ± 0.091	0.295 ± 0.085

Thus, the differences between the series are not very large absolutely,

TABLES 12-16 (continued)

Data pertaining to depression of the freezing point and osmotic pressure
Table 15, Series D

Sample number	Depression of the freezing point			Osmotic pressure in atmospheres		
	Wall	Prolifcation	Difference	Wall	Prolifcation	Difference
304	0.609°	0.588°	-0.021°	7.336	7.084	-0.252
305	0.617°	0.573°	-0.044°	7.433	6.903	-0.530
310	0.596°	0.550°	-0.046°	7.180	6.626	-0.554
311	0.599°	0.560°	-0.039°	7.216	6.747	-0.469
315	0.622°	0.560°	-0.062°	7.493	6.747	-0.746
316	0.615°	0.586°	-0.029°	7.409	7.059	-0.350
321	0.616°	0.544°	-0.072°	7.421	6.554	-0.867
324	0.606°	0.557°	-0.049°	7.300	6.710	-0.590
328	0.570°	0.550°	-0.020°	6.867	6.626	-0.241
329	0.596°	0.577°	-0.019°	7.180	6.951	-0.229
330	0.638°	0.572°	-0.066°	7.685	6.891	-0.794
347	0.633°	0.587°	-0.046°	7.625	7.072	-0.553

Table 16, Series E

21	0.578°	0.549°	-0.029°	6.963	6.614	-0.349
24	0.604°	0.568°	-0.036°	7.276	6.843	-0.433
28	0.639°	0.598°	-0.041°	7.697	7.204	-0.493
40	0.551°	—	—	6.638	—	—
41	0.545°	0.520°	-0.025°	6.566	6.265	-0.301
102	0.667°	0.603°	-0.064°	8.034	7.264	-0.770
103	0.659°	0.594°	-0.065°	7.938	7.156	-0.782
118	0.624°	0.596°	-0.028°	7.517	7.180	-0.337
119	0.649°	0.596°	-0.053°	7.818	7.180	-0.638
131	0.704°	0.666°	-0.038°	8.479	8.022	-0.457
141	0.696°	0.648°	-0.048°	8.383	7.806	-0.577
146	0.612°	0.546°	-0.066°	7.372	6.578	-0.794
147	0.627°	0.540°	-0.087°	7.553	6.506	-1.047
153	0.627°	—	—	7.553	—	—
364	0.593°	0.554°	-0.039°	7.144	6.674	-0.470
365	0.648°	0.623°	-0.025°	7.806	7.505	-0.301
377	0.613°	0.590°	-0.023°	7.384	7.108	-0.276
392	0.645°	0.618°	-0.027°	7.770	7.445	-0.325
407	0.590°	0.576°	-0.014°	7.108	6.939	-0.169
408	0.702°	0.663°	-0.039°	8.455	7.986	-0.469

though on the whole probably significant in comparison with their probable errors.

The range of variation in *osmotic pressure* is shown in the accompanying summary:

Experiment	Wall	Prolifcation
A	7.22 — 10.15 = 2.93	6.41 — 9.73 = 3.32
B	7.27 — 8.76 = 1.49	7.12 — 8.46 = 1.34
C	7.26 — 8.39 = 1.13	6.33 — 7.84 = 1.51
D	6.87 — 7.69 = 0.82	6.55 — 7.08 = 0.53
E	6.57 — 8.48 = 1.91	6.27 — 8.02 = 1.75

Notwithstanding the considerable period of time over which these collections were made, and the possibility that some of the more extreme values may be due to some undetected error of determination, the range of osmotic pressure is actually rather narrow; though, when considered in comparison with the mean pressure, it is relatively rather wide. Expressing the results in the more logical terms of standard deviation, we find:

Experiment	Wall	Proliferation
C	0.304 ± 0.039	0.363 ± 0.046
D	0.211 ± 0.029	0.180 ± 0.025
E	0.510 ± 0.057	0.488 ± 0.055

The collections from Series E, which were made over a wide range of time and conditions, are distinctly more variable. This conclusion, based on the standard deviation, is substantiated by the determination of the coefficients of variation.

With regard to the relative conditions in the wall and the included mass, the results of this measurement of the concentration of non-electrolytes, and dissociated and non-dissociated electrolytes, are in close agreement with those for electrolytes alone, as measured in terms of conductivity. There is a lower osmotic pressure in the sap of the included mass than in the ovary wall. The great majority of these differences are of sufficient magnitude to be certainly significant. The data here are very consistent. Only three exceptions are found: in two the proliferation has a greater osmotic pressure than has the sap from the ovary wall; in one the results are identical.⁹

Turning to the summary giving the *mean* osmotic pressure for wall and abnormal tissue, and their differences, we note that the osmotic pressure of the sap of the included mass is from one half to one atmosphere lower than that of the wall of the fruit in which it is produced. Expressing the difference in percentages, as in the case of conductivity above and using as a base the mean for the ovary wall, we find that the osmotic pressure is 12.1, 7.0 and 6.5 percent lower in the mass. These relative values are distinctly

⁹ These three cases occur in one of the two small series. No explanation can be given.

lower than those for electrolytes, which were 34.7, 34.6 and 29.6 percent.

These facts seem to indicate that the differentiation between fruit wall and included mass due to electrolytes tends to be strongly reduced by the non-electrolytes. We noted that the difference for specific gravity was only from 5.50–11.50 percent, as compared with 30.00–35.00 percent for electrolytes. This may be taken as evidence in the same direction as the findings for osmotic pressure.

D. RATIO OF ELECTRICAL CONDUCTIVITY TO DEPRESSION OF THE FREEZING POINT. (Data, Tables 7–11.) We have desired to determine the amounts of mineral salts and organic constituents of the sap, but because of the great technical difficulties have been unable to do so for a series of materials as extensive as those with which we have had to deal. We have therefore contented ourselves with the determination of the ratio of the conductivity to the depression of the freezing point. While these are not measures in the same terms, a comparison of the ratios in such closely related materials as the tissues of the ovary wall and of the abnormal mass is perhaps quite legitimate.

This ratio is given in our tables as κ/Δ . On the assumption that nearly all of the electrical conductivity is due to dissociated inorganic salts, such a ratio shows the relative proportion of salts in the total solutes. The results are remarkably consistent. In only one sample does the difference between the ratios possess a positive sign. Thus the organic substances form a greater and the inorganic a smaller proportion of the solutes in the sap of the proliferation than do those in the sap of the ovary wall. The averages for the three larger series are given below:

Experiment	Wall	Proliferation	Difference
C	0.002409 ±0.000018	0.001800 ±0.000046	-0.000609 ±0.000049
D	0.002045 ±0.000031	0.001438 ±0.000019	-0.000607 ±0.000036
E	0.002164 ±0.000036	0.001642 ±0.000034	-0.000522 ±0.000050

The constants for comparable tissues are in good general agreement from series to series, but the differences though actually very

TABLES 17-21

Data pertaining to concentration and mean molecular weights of solutes in the saps

Table 17, Series A

Sample number	Concentration of solutes in the plant sap			Mean molecular weight of solutes		
	Wall	Proliferation	Difference	Wall	Proliferation	Difference
76 + 86	0.04294	0.03792	-0.00502	94.7	88.7	- 6.0
90	0.03966	0.03774	-0.00192	87.9	86.9	- 1.0
207	0.03798	0.03423	-0.00375	117.0	119.7	+ 2.7
292	0.03767	0.03579	-0.00188	116.8	116.8	± 0.

Table 18, Series B

I	0.04069	0.03991	-0.00078	122.7	122.3	- 0.4
2 + 5	0.04312	0.04600	+0.00288	132.6	130.2	- 2.4
34	0.04618	0.04617	-0.00001	141.7	141.7	± 0.
35	0.04824	0.04537	-0.00287	146.1	134.6	-11.5
64	0.04244	0.03945	-0.00299	108.4	104.4	- 4.0
189	0.04136	0.03571	-0.00565	119.8	118.8	- 1.0
286	0.03997	0.03817	-0.00180	123.1	120.1	- 3.0

Table 19, Series C

210	0.04630	0.04365	-0.00265	123.6	124.7	+ 1.1
211	0.04152	0.03723	-0.00429	119.4	118.2	- 1.2
213	0.03922	0.03731	-0.00191	118.2	125.3	+ 7.1
214	0.04176	0.03513	-0.00663	120.6	118.0	- 2.6
221	0.04449	0.03838	-0.00611	119.6	117.0	- 2.6
238	0.04123	0.03394	-0.00729	117.6	112.9	- 4.7
245	0.03848	0.03740	-0.00108	115.4	125.3	+ 9.9
246	0.04173	0.03491	-0.00682	120.7	119.1	- 1.6
247	0.03955	0.03294	-0.00661	113.2	109.6	- 3.6
248	0.03760	0.03309	-0.00451	116.0	117.0	+ 1.0
260	0.03928	0.03445	-0.00483	114.5	113.4	- 1.1
261	0.03781	0.03525	-0.00256	111.1	116.9	+ 5.8
262	0.04123	0.03363	-0.00760	118.2	114.1	- 4.1
269	0.03889	0.03543	-0.00346	108.1	112.8	+ 4.7

small are several times as large as their probable errors. Thus, the difference between the ratio for exp. C and D is 0.000364 ± 0.000036 , that between C and E is 0.000245 ± 0.000040 , that between D and E is 0.000119 ± 0.000048 . The mean difference between the constants for the ovary wall and the included mass is in all cases over ten times as large as its probable error, and so unquestionably significant.

E. MEAN MOLECULAR WEIGHT OF THE SOLUTES IN THE SAP. (Data, Tables 17-21.) The results for mean molecular weight of the solutes is much less consistent from sample to sample than any other measure here discussed. This is to be expected from the technical difficulties in its determination. As we have computed

TABLES 17-21 (continued)

Data pertaining to concentration and mean molecular weights of solutes in the saps

Table 20, Series D

Sample number	Concentration of solutes in the plant sap			Mean molecular weight of solutes		
	Wall	Proliferation	Difference	Wall	Proliferation	Difference
304	0.04000	0.03909	-0.00091	122.2	123.7	+ 1.5
305	0.04017	0.03688	-0.00329	121.1	119.7	- 1.4
310	0.03745	0.03469	-0.00276	116.9	117.3	+ 0.4
311	0.03671	0.03487	-0.00184	114.0	115.8	+ 1.8
315	0.04117	0.03776	-0.00341	123.1	125.4	+ 2.3
316	0.04035	0.03893	-0.00142	122.0	123.6	+ 1.6
321	0.03841	0.03702	-0.00139	116.0	126.6	+10.6
324	0.03907	0.03692	-0.00215	120.0	123.3	+ 3.3
328	0.03742	0.03518	-0.00224	122.1	119.0	- 3.1
329	0.04080	0.03817	-0.00263	127.3	123.0	- 4.3
330	0.04055	0.03706	-0.00349	118.2	120.5	+ 2.3
347	0.04103	0.03808	-0.00295	120.6	120.7	+ 0.1

Table 21, Series E

21	0.04338	0.03890	-0.00448	129.8	131.8	+ 2.0
24	0.04388	0.04186	-0.00202	135.1	137.1	+ 2.0
28	0.04740	0.04684	-0.00056	138.0	145.7	+ 7.7
40	0.03968	—	—	133.9	—	—
41	0.03580	0.03380	-0.00200	122.2	120.9	- 1.3
102	0.04214	0.03904	-0.00310	117.5	120.4	+ 2.9
103	0.04303	0.03850	-0.00453	121.5	120.6	- 0.9
118	0.03755	0.03676	-0.00079	111.9	114.7	+ 2.8
119	0.04016	0.03718	-0.00298	115.1	116.0	+ 0.9
131	0.04652	0.04442	-0.00210	122.9	124.1	+ 1.2
141	0.04618	0.04403	-0.00215	123.4	126.4	+ 3.0
146	0.03705	0.03422	-0.00283	112.6	116.6	+ 4.0
147	0.03871	0.03332	-0.00539	114.8	114.8	± 0
153	0.04189	—	—	124.3	—	—
364	0.03763	0.03650	-0.00113	118.0	122.6	+ 4.6
365	0.04355	0.04305	-0.00050	125.0	128.5	+ 3.5
377	0.04170	0.04149	-0.00021	126.5	130.8	+ 4.3
392	0.04652	0.04441	-0.00211	134.2	133.7	- 0.5
407	0.04004	0.03908	-0.00096	126.2	126.2	± 0
408	0.04899	0.04818	-0.00081	129.8	135.2	+ 5.4

it, three laboratory operations are involved: (a) the determination of specific gravity by weighing in a pycnometer; (b) the determination of total solids by drying a pipetted sample (10 c.c.) at 100°; and (c) the measurement of the depression of the freezing point. A slight error in any of these processes would influence the final constant. Even in work with isolated chemical substances the determination of exact molecular weights by cryoscopic methods presents difficulties. The close agreement in the observed mean molecular weights is, we believe, a good criterion of the care with which our analyses have been carried out.

The ranges in mean molecular weight of the solutes for the several experiments are :

Experiment	Wall	Prolification
A	87.9 — 117.0 = 29.1	86.9 — 119.7 = 32.8
B	108.4 — 146.1 = 37.7	104.4 — 130.2 = 25.8
C	108.1 — 123.6 = 15.5	112.8 — 125.3 = 12.5
D	114.0 — 127.3 = 13.3	115.8 — 126.6 = 10.8
E	111.9 — 138.0 = 26.1	114.7 — 145.7 = 31.0

The differences in the ranges are quite striking. For both ovary wall and abnormal carpellary mass, exp. C and D show a far narrower range of variation than the other three series, notwithstanding the fact that exp. A and B, which show on the whole the widest ranges, comprise but a few determinations.¹⁰

For the three large series the standard deviations and coefficients of variation are :

Series	Standard deviation	Coefficient of variation
C		
Wall	4.000 ± 0.510	3.423
Prolification	4.718 ± 0.601	4.017
D		
Wall	3.434 ± 0.473	2.855
Prolification	3.125 ± 0.430	2.571
E		
Wall	7.561 ± 0.850	6.118
Prolification	8.371 ± 0.941	6.649

Thus, the variation in the mean molecular weight in exp. E, as measured by the standard deviation or coefficient of variation, is distinctly higher than that of the two other large series, just as it appeared to be when measured by the range of variation. The explanation of this greater variability in certain of the collections is probably the same as that suggested for the observed differences in other constants—that of environmental heterogeneity and of differences in the physiological state of the individual at the time the samples were taken.

¹⁰ The significance of this fact must be obvious after a moment's consideration. If differences in constants were to be attributed to faulty technique, one would expect to find the greatest differences in the largest series, for there would be more opportunities for errors leading to widely divergent constants.

There seems to be some evidence in favor of the conclusion that the mean molecular weight of the solutes from the abnormal structure is higher than that from the ovary wall. There are 29 cases in which the mean molecular weight for the included mass is higher than that for the ovary wall, to 22 cases in which the reverse is true. In four instances the two values are identical. The averages for the three major series of determinations are as follows:

Experiment	Wall	Proliferation	Difference
C	116.87 ± 0.72	117.45 ± 0.85	+0.58 ± 1.11
D	120.29 ± 0.67	121.55 ± 0.61	+1.26 ± 0.90
E	123.58 ± 1.20	125.89 ± 1.33	+2.31 ± 1.79

In all cases the averages for the solutes of the proliferous mass are higher than for those of the fruit wall, but the probable error of the difference is so high that little weight is to be attached to it.

While these results are by no means conclusive they seem to indicate that the solutes in the sap of the proliferous mass have a slightly greater average molecular weight than the solutes in the sap of the ovary wall. Such a conclusion would be in good agreement with the result noted under (*D*) above, *i. e.*, that the organic materials form a greater proportion of the dissolved substances in the sap of the proliferation than they do in the sap of the ovary wall. It is but reasonable to suppose that the organic substances have a greater molecular weight than have the ions and undissociated molecules of the inorganic salts.

5. Summary and conclusions. In *Passiflora gracilis* proliferation of the fruit frequently occurs. This generally consists in the production of a series of whorls of incompletely closed carpels borne on a short stalk arising from the bottom of the fruit cavity, and represents in all probability a continuation of the main axis which gave rise to the carpels entering into the composition of the fruit wall.

As one of the steps in the analysis of the factors to which this highly remarkable teratological variation is due, we have had under investigation for some time the physico-chemical properties of the cell sap of the normal and teratological fruits, and of the parts of the teratological fruit.

In this paper we present data concerning the physico-chemical properties of the cell sap from the tissues of the wall and the abnormal carpellary whorl¹¹ of fruits with trimerous fruit wall and tetramerous¹ proliferation. The materials comprise about 60 samples of this class obtained by the dissection of about 100,000 fruits.

The following conditions have been found in the materials examined.

The physico-chemical constants considered—*specific gravity, concentration, depression of the freezing point, osmotic pressure, electrical conductance and mean molecular weight*—are all susceptible to the influence of the environmental and possibly to the physiological state of the plant upon which they are borne. This is shown by the facts (*a*) that the mean values of the constants differ sensibly from series to series;¹² and (*b*) that the variability of the determinations within the individual cultures is, in general, larger in the cases in which the collections extended over a longer period of time, and in consequence comprised fruits which had been developed under a wider range of conditions.

The *specific gravity* of the sap of the fruit wall is of the order 1.0200, while that of the proliferous mass is distinctly lower. Concentration, *i. e.*, *weight of solutes / weight of water*, is about 0.0400 for the wall and sensibly lower for the included mass. If the differences in specific gravity of the sap from the wall and the included mass be expressed in percentage ratios, with the variable term (*d-1*) of the specific gravity of the wall as a base, the density of the sap from the mass is, for the averages of the larger series, 5.5, 5.7 and 11.6 percent lower than that of the wall.

The specific *electrical conductivity* is of the order 0.013000 for the sap of the ovary wall, and about 0.004300 lower for that extracted from the included carpellary whorl. In the three principal series of determinations the conductivity of the sap from the in-

¹¹ The two parts of the abnormal fruit discussed here are the normal outer wall, the wall, and the included abnormal whorl of carpels, called the proliferation or the included mass.

¹² Possibly these differences are in part due to differentiation in the strains of plants used; two of the five series were from a source different from the others.

cluded mass is 29.6, 34.7 and 34.6 percent lower than that from the wall.

The *osmotic pressure* of the extracted sap, as determined by the *depression of the freezing point*, is, in round numbers, 7.50 atmospheres in the fluids of the wall and roughly half an atmosphere less in the proliferation. For the three large series the mean constants for the abnormal whorl are 12.1, 7.0 and 6.5 percent lower than the values for the fruit wall, which serves as a base for the calculation of the percentages.

The difference between the mean *molecular weight of the solutes* in the sap of the wall and the abnormal mass, as determined by

$$\frac{\text{Weight of solids}}{\text{Weight of water}} \times \frac{1860}{\Delta},$$

is the most variable from sample to sample of any of the constants. Possibly this is due, to some extent, to the fact that an unavoidable experimental error, in any one of three distinct laboratory operations involved in the determination of this constant, would influence the final result. However, the slight absolute variation in mean molecular weight seems to us to indicate a great reliability of the constants.

The mean molecular weight is of the order 120. There is some evidence that this constant is somewhat higher for the solutes of the abnormal tissues than for those of the fruit wall.

The problem of the *relative abundance of electrolytes* in the normal and abnormal tissue is apparently of considerable importance, but is surrounded with serious technical difficulties. The *ratio of electrical conductance to depression of the freezing point*, κ/Δ , is significantly higher in the sap from the wall than in that from the abnormal tissue. This indicates that the concentration of electrolytes is relatively higher in the fruit wall, while that of non-electrolytes is higher in the sap of the teratological structure.

Other evidences support the immediately foregoing conclusion. Thus, the fact that the percentage difference between the sap from the wall and that from the abnormal whorl of carpels is far larger in the case of conductivity than for osmotic pressure, or specific gravity, indicates that the differentiation of these two sets of tis-

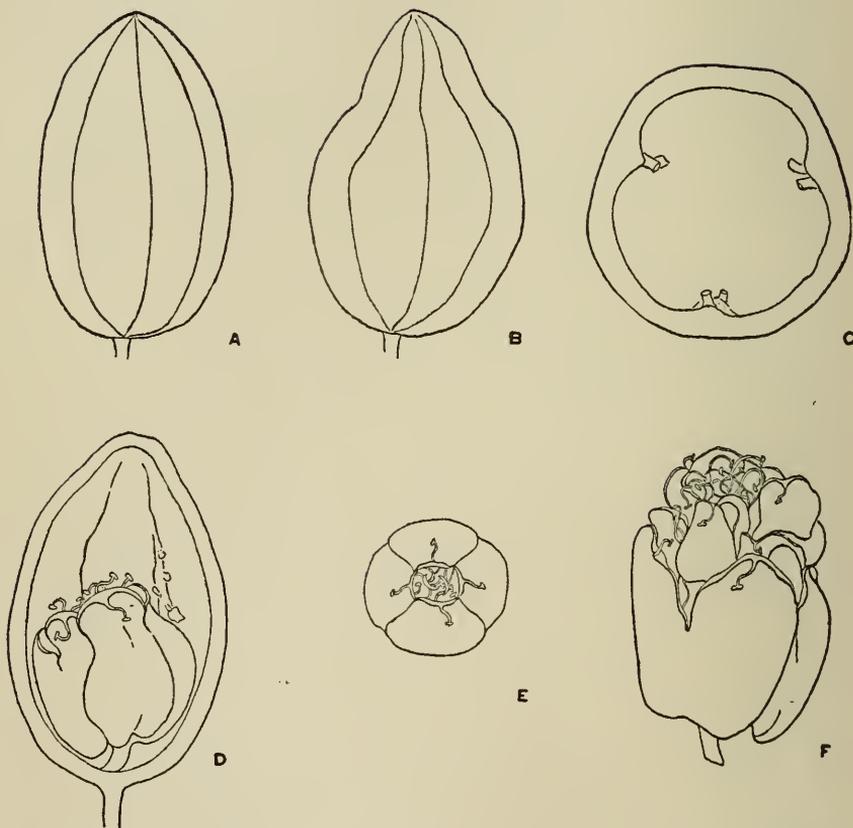
sues is greater with respect to electrolytes than to non-electrolytes. Again, the apparently higher mean molecular weight of the solutes from the abnormal tissues evidences in favor of the presence of greater quantities of non-dissociated substances.

In conclusion we should like to state that this paper is purposely limited strictly to the presentation of facts. That the statements concerning matters of fact are sound is, we believe, established by the high degree of consistency in the results of numerous physico-chemical determinations based on very large masses of biological material.

We feel that in the present state of our knowledge an attempt to discuss the physiological significance of these results is premature. For the present, the imperative need of physiology is sound quantitative measurements of essential variables, and harm rather than good is done by premature theoretical discussions.

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HARRIS, GORTNER AND LAWRENCE: STUDIES ON THE PHYSICO-CHEMICAL PROPERTIES OF VEGETABLE SAPS

EXPLANATION OF PLATE 3

Diagrams illustrating the nature of the type of proliferation of the fruit of *Passiflora gracilis*, considered in this paper.

(A) Form of the normal fruit, showing three of the six external sutures.

(B) External appearance of extreme case of proliferation, showing the wall distended by the large included mass.

(C) Cross section of the fruit wall, showing the three placentae, with funiculi, from which the seeds have been detached. Externally these are represented by three sutures. Three other sutures alternate with these, completing the six which are found in the normal fruit and in this type of proliferation.

(D) Longitudinal half of a proliferous fruit, showing one placenta and the line of one of the intermediate (dorsal) external sutures, which is but faintly visible internally. The stalked proliferation with three of the four external carpels visible is of typical size. With the exception of the presence of the proliferation, the diagram is quite typical of the normal fruit.

(E) A rather small carpellary mass seen from above, exhibiting the pronounced tetramery of the included mass in fruits of this class.

(F) Unusually large proliferation, showing the internal whorls of carpels greatly developed and projecting beyond the tetramerous whorls, of which three members are visible. In figures D, E, and F, note the well developed styles and stigmas. All figures are about twice natural size.

COMPARISONS OF URINARY AND SERUM FINDINGS IN THE DIAGNOSIS OF TUBERCULOSIS*

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INTRODUCTION. The urinary changes during tuberculosis have been given considerable study. The results obtained by different authors contradict each other, however, in many respects. It is very likely that the disparities among the results of different authors are chiefly due to the fact that it is very difficult to select, for such studies, a group of tuberculous individuals wholly free from conditions that influence the urinary findings and thus complicate the problem. From a review of the literature it appears, however, that a number of investigators have been in accord in finding marked increases of neutral sulfur in the urines of tuberculous individuals. These findings appeared to be very promising from the standpoint of diagnosis, because they seemed to offer the modified procedure of Moritz Weiss as a substitute for the original technic of Ehrlich.

Beginning with Moritz Weiss,^{1,2} a number of investigators have found the neutral-sulfur test very helpful in the diagnosis of tuberculosis. They have claimed, for instance, that the amount of neutral sulfur increases with the progress of the disease and that, in general, the amount of neutral sulfur in the urine suggests the degree of tissue destruction in the body.³ According to Moritz Weiss, the persistence of neutral sulfur in, or its disappearance from, the urine, in the progress of anti-tuberculous treatment, indicates the degree of efficiency of the treatment. Curti,⁴ in repeating the work of Weiss, found not only that this test was constantly

* Proceedings of the Columbia University Biochemical Association, Dec. 4, 1914; *BIOCHEM. BULL.*, 1915, iv, p. 211.

¹ M. Weiss: *Wien. klin. Woch.*, 1913, p. 1705.

² M. Weiss and A. Weiss: *Ibid.*, 1912, p. 1183.

³ Keim and Vigot: *Presse medicale*, 1914, p. 153.

⁴ Curti: (quoted in) *Münch. med. Woch.*, 1914, p. 1577.

positive in clinical tuberculosis, but also that, in a number of cases of pneumothorax, the quantity of neutral sulfur ran parallel with other clinical symptoms of the disease, and always became negative in cases which could be considered clinically cured. Tecon and Aimart,⁵ Jaquerod,⁶ Kaplansky,⁷ and others, have also found the presence of excess of neutral sulfur, in urine, of diagnostic as well as prognostic value in tuberculosis.

In studying the question of serum diagnosis of tuberculosis in this hospital, we have had a number of cases in which positive serum findings were the only symptoms of possible infection, all other data, except the Von Pirquet test, speaking against tuberculosis. We naturally thought of the possibility of controlling such positive findings with other tests. Since, according to the authors cited above, the presence of neutral sulfur in urine is of high diagnostic value, we compared our serological findings, in a number of cases, with those obtained in the examination of the urines.

From the very nature of the test for neutral sulfur, it is evident that it cannot be specific for tuberculosis; on the contrary, it is quite constant in typhoid, for instance. In order to introduce the necessary corrections in the final results, we decided to ascertain, by first examining at random a number of cases, what conditions, besides those of tuberculosis and typhoid, favor the occurrence of an excess of neutral sulfur in the urine.

TECHNIC AND RESULTS. In the preliminary examinations, as well as in the later ones, both the Ehrlich and the Weiss procedures were used in parallel tests.

Diazo test. The reagents for this test, consisting of (a) 0.5 percent sol. of sodium nitrite and (b) a sol. of 5 gm. of sulfanilic acid in 50 c.c. of conc. hydrochloric acid sol. diluted to 1000 c.c. with distilled water, are kept in separate stocks. Of these reagents, 50 parts of the sulfanilic acid sol. and 1 of the sodium nitrite sol. are freshly mixed for use in the test.

An equal volume of this freshly mixed reagent is added to 5 c.c. of urine and, after shaking, an excess of strong ammonium hy-

⁵ Tecon and Aimart: *Presse medicale*, 1914, p. 131.

⁶ Jaquerod: Discussion, *ibidem*.

⁷ Kaplansky: Discussion, *ibidem*.

droxid sol. is added. In the case of a positive reaction, both the fluid and the foam turn red.

Moritz Weiss test. To 5 c.c. of urine 10 c.c. of water are added, in order to reduce the intensity of the color. This dilute urine is then treated with 3 drops of 0.1 percent sol. of potassium permanganate. In the presence of an excess of neutral sulfur, a deep yellow color appears. The reaction is very delicate, but the reading is often made difficult by the deep yellow color of certain specimens of urine, and also because a number of normal urines become more yellowish after addition of permanganate.

In the first group of tests, 172 cases were examined at random, irrespective of clinical diagnosis or history. The comparative results are given in Table I.

TABLE I

Data pertaining to the diazo and Weiss tests for neutral sulfur in normal and abnormal urines

Diagnosis	Diazo test		Weiss test		Number of cases	Percentage of positive reactions	
	+	-	+	-		Diazo test	Weiss test
Typhoid.....	5	9	6	8	14	38.1	52.3
Clinical tuberculosis.....	3	4	5	2	7		
Lues.....	3	13	3	12	15	17.8	21.8
Diabetes.....	1	0	1	0	1		
Nephritis.....	1	5	3	3	6		
Cholecystitis.....	1	4	2	3	5		
Pneumonia.....	0	1	1	0	1		
Cancer.....	0	4	0	4	4		
Hypothyroidism.....	0	2	0	2	2		
Gangrene.....	1	0	1	0	1		
Pleural effusion.....	1	0	1	0	1		
Surgical, with pus ¹	2	22	4	20	24		
Pregnancy, normal.....	1	16	1	16	17		
Eclampsia.....	0	2	1	1	2		
Unclassified.....	16	16	12	20	32		
Normal ²	1	39	3	37	40		
Total.....	35	137	44	128	172	20.35	25.58

¹ Including appendicitis, salpingitis, pus tubes, etc.

² Included under "normal" are conditions in which there is a certain degree of systemic disturbance, *e. g.*, fractures, hernia, burns, concussions, etc.

It is evident, from the data in Table I, that over 20 percent of the cases taken at random in the hospital gave positive tests for neutral sulfur in the urine. In order to determine the diagnostic

value of these findings and assuming that, in tuberculosis and in typhoid, neutral sulfur is usually present in the urine, we examined the histories of all the cases in which positive tests were obtained by either method and thus eliminated these two diseases as the cause of the appearance of neutral sulfur in the urine, with the following results:

	Total No.	Diazo+ Weiss+	Diazo+ Weiss-	Diazo- Weiss+	Diazo- Weiss-
Tuberculosis or typhoid in the history or diagnosis.....	50	27	2	11	10
No tuberculosis nor typhoid in history or diagnosis.....	122	4	2	3	113

As this summary shows, of 122 cases in which the history of tuberculosis and typhoid were ruled out, 113 gave negative tests by both methods. The 4 cases in which both diazo and Weiss tests were positive were clinically cases of diabetes, cholecystitis, gangrene and pleural effusion. Whether in these conditions, the findings for neutral sulfur are constant we cannot tell, for we had only one case of each in this series.

Comparisons of urinary findings with the results of the serum test. Having thus ascertained the degree of efficiency of the tests as compared with each other, and having found that the tests are usually negative for cases where both typhoid and tuberculosis are excluded, we proceeded to compare the findings of the urinary examination with those of the serum test.

TABLE 2

Data pertaining to comparative urinary findings and results of the serum test

		Total No.					Percentage of positive findings		
			Diazo + Weiss +	- - Weiss	Diazo + Weiss +	Diazo - - Weiss -	Both tests.	Diazo test.	Weiss test.
Clinically tuberculous. . .	Serum test: Positive. . .	92	40	3	7	42	43.5	46.7	51.1
	Serum test: Negative. . .	8	6	0	2	0	74.4	74.4	100
Clinically non-tuberculous	Serum test: Positive. . .	11	1	0	1	9	9.1	9.1	1.20
	Serum test: Negative. . .	89	12	2	6	69	13.5	15.7	2.25
Total.		200	59	5	16	120	28.	30.5	37.8

The serum test was that described in detail before.⁸ The antigen was that of Besredka.⁹ The total number of cases compared was 200. The results are given in Table 2.

It was previously shown by one of us¹⁰ that, in cases of certain tuberculosis, only 90–95 percent of the cases give positive serum reactions. It was also shown that, in the advanced cases, the reaction is usually absent, so that it was even suggested that a failure of the serum test in an advanced case of tuberculosis may be taken as a bad prognostic sign.

Comparing the earlier findings with those of the present series, we notice the same phenomenon, namely, in 8 out of 100 cases the serum reaction is negative in spite of the fact that the cases are undoubtedly tuberculous. The value attributed previously to these negative findings seems to be justified by the present results, in spite of the fact that the urinary findings appear to contradict the serological, inasmuch as out of 92 cases of positive serum results the urinary findings in 42 cases, at least, were negative by both methods, and in only 40 cases, or less than 50 percent, the urinary findings confirmed, by both methods, the serum findings. On the other hand, out of 8 cases of tuberculosis in which serum findings were negative, every case gave a positive Weiss test and 6 cases also gave positive diazo tests.

Remembering that the presence of neutral sulfur, according to Weiss, is due to the destruction of tissue, and that the intensity and frequency of the occurrence of the reaction run parallel with the progress of the disease, the findings above may be of great value in confirming the opinion, stated earlier, that cases of tuberculosis in which there is no circulating antibody are cases in which there is considerable destruction of tissues, as indicated by the excess of sulfur in the urine.

The finding of 11 positive serum reactions among the cases which do not present any symptoms of tuberculosis clinically, should not be attributed, as was explained before,¹⁰ to non-specificity of serum diagnosis, but rather to the fact that in its earlier stages,

⁸ Bronfenbrenner: *Zeitschr. f. Immunitätsforsch.*, 1914, xxiii, p. 221.

⁹ Besredka and Manouschine: *Compt. rend. soc. biol.*, 1914, lxxvi, p. 180.

¹⁰ Bronfenbrenner: *Arch. of Intern. Med.*, 1914, xiv, p. 786.

the tuberculous process does not induce symptoms enough for clinical diagnosis. A positive serum test in such cases may indicate its extreme diagnostic value. Although urinary findings in these 11 cases were all negative, with the exception of one, which was a case of typhoid, they indirectly explain the failure of clinicians promptly to discover the tuberculous process, since in incipient cases the destruction is so insignificant, that no increase of sulfur can be detected.¹¹

SUMMARY. Comparisons of the diagnostic values of the urinary findings for neutral sulfur with those for the serum reaction in tuberculosis reveal the following facts:

1. The diazo or Weiss test in tuberculosis is less constant, in general, than the serum reaction.

2. Positive results with the diazo or the Weiss test are of value only if typhoid is excluded. There are also a few other pathological conditions in which these tests are positive, but the data at hand are inadequate for conclusions regarding the constancy of these findings.

3. The urinary findings are not sufficiently frequent in tuberculosis to be of special diagnostic value, even when other possible complications giving rise to positive tests can be excluded.

4. The occurrence of increased amounts of urinary neutral sulfur, in advanced stages of disease, is quite constant and may be of prognostic value, especially in connection with corresponding negative findings in the serum.

¹¹ These examinations were made during the spring and summer of 1914. At that time we had the opportunity of making the tuberculin test on only 6 cases of the 11 reported above. Since then, however, in two more cases the tuberculin test was made and in all 8 cases it was positive. Moreover, very recently we received a report that one of the patients of this series had a hemorrhage and also has a very distinct consolidation at present—about 8 months after the first serum test. We take this opportunity to thank Dr. Marks, who was kind enough to give us this information.

THE ROLE OF SERUM ANTI-TRYPSIN IN THE ABDERHALDEN TEST*

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In previous communications (1, 2) we outlined the mechanism of the Abderhalden test as an autodigestion of serum protein of the patient due to the removal of antitryptic inhibition. This removal of anti-trypsin, although quite apparent in our experiments, has not thus far been demonstrated directly. In this preliminary report we wish to record the fact that actual measurement of the anti-trypsin in the serum, before and during the progress of the Abderhalden reaction, reveals the fact that the anti-tryptic titer of the serum is actually involved. The diminution of the anti-tryptic activity of the serum, as tested against trypsin solution, takes place in a specific manner, inasmuch as it occurs only in cases where the serum used is that of pregnant individuals and is parallel with the intensity of the Abderhalden test; so that the estimation of anti-trypsin in serum undergoing digestion, after its removal from contact with placenta, may be used as a method of diagnosis of pregnancy parallel with, and complementary to, that of the Abderhalden test. Moreover, it is evident that this inactivation of anti-trypsin takes place at ice-box temperature as well as at the temperature of the incubator. If the Abderhalden test is divided into two periods, as was shown before (3), over 30 percent of the anti-trypsin is removed during the first part of the reaction.

The comparison of the anti-tryptic index of the serum, before and during the Abderhalden test, with the index obtained by measuring the effect of kaolin and other substances capable of adsorbing anti-trypsin in a non-specific manner, confirms our contention (1) that the appearance of dialyzable cleavage products in serum may be determined by specific as well as by non-specific mechanisms, and that the essential part of this phenomenon is the removal of serum anti-trypsin, which in turn liberates the normal proteases of the serum, thus setting the serum into autodigestion.

* Proceedings of the Columbia University Biochemical Association, Dec. 4, 1914; *BIOCHEM. BULL.*, 1915, iv, p. 211.

¹ Bronfenbrenner: *Proc. Soc. Exp. Biol. and Med.*, 1914, xii, pp. 4 and 7.

² Bronfenbrenner: *Jour. Exp. Med.*, 1915, xxi, p. 221.

³ Bronfenbrenner, Mitchell and Schlesinger: *BIOCHEM. BULL.*, 1914, iii, p. 386.

ON THE NATURE OF THE ABDERHALDEN¹ REACTION¹

J. BRONFENBRENNER

According to Ehrlich's theory the parenteral introduction of foreign protein causes the cells of the body to produce an excess of specific receptors, which, at a certain period of the process, circulate freely in the blood, and are known to the student of immunity under the name of amboceptors, antibodies, or *substances sensibilisatrices*. These specific antibodies are complex in character; and, although they are directly responsible for the specificity of the protective processes in the body, they are not of themselves active principles. It is to complement that Ehrlich and his school attribute the power of action on antigen.

The properties of the antibodies resemble those of enzymes in very few respects, while they differ from them at many points. According to Abderhalden, however, the parenteral introduction of foreign protein results in the production of specific enzymes capable of directly digesting antigen *in vitro*. I disagree with those who think that Abderhalden has proved that these substances are enzymic in character. On the one hand it is difficult to believe that the organism is able to supply so many specific enzymes; on the other, it is improbable that the enzymes circulating in the blood are strong enough to digest coagulated protein, as is the case in the Abderhalden test.

In our own experiments we tried to produce a specific enzyme by repeated inoculation of rabbits with egg-white; and, although the serum of these animals contained a very large amount of antibodies, a Mett-tube filled with coagulated egg-white failed to show even the slightest traces of digestion of the egg-white after suitable immersion in such serum. This and the results of other experiments led us to conclude that either the enzymes on which the supposed di-

¹ Discussion at a meeting of the Pennsylvania State Medical Society, at Pittsburgh, September 22, 1914. See also Proceedings of the Columbia University Biochemical Association, Dec. 4, 1914; *BIOCHEM. BULL.*, 1915, iv, p. 211.

gestion of placenta depends in the Abderhalden test are essentially different from the substances obtained by immunisation of rabbits, or that they are both alike, but not enzymic in character. Further experiments along this line convinced us that the latter alternative is the correct one. We corroborated the earlier findings of Stephan and Hauptmann, that the complement plays an important part in the Abderhalden test, but also found that the specific enzymes (so-called) of Abderhalden behave, in every way, like antibody, as understood in the terminology of immunity. We also succeeded in exhausting the serum of pregnant individuals of its specific elements, and in actually sensitizing placenta-protein so as to obtain a positive ninhydrin test after the addition of fresh human or animal (male or female) serum.

Having thus convinced ourselves that the Abderhalden test did not depend on any enzyme specifically able to digest placenta-protein (since the addition of any serum favored a positive ninhydrin test, provided the serum was added to previously sensitized placenta) we concluded that the ninhydrin test is nothing but a new expression of the phenomenon which previously had been brought to light by the indicator of Bordet-Gengou, viz., hemolysis. Viewed in this light the Abderhalden test, without offering anything new on the theory or mechanism of immunity, introduces a very effective indicator of the occurrence of the reaction.

As to the mechanism of the test proper, I wish to state without going into the details of our experiments, that I have proof of the fact that in the Abderhalden test placenta is not digested, but that the amino-acids and polypeptids, which dialyse through the wall of the thimble, come from the serum. I have noted their appearance in a serum after it had been incubated with placenta-protein for some time, and under certain conditions. These dialysable products result from the digestion of the globulin in the serum by the accompanying serum protease; in other words, as a result of the autodigestion of the patient's serum.

The proteolytic ferment responsible for this auto-digestion is not specific, but is present in all fresh sera, *in vivo* as well as *in vitro*. The action of this ferment, while in the body, is arrested by the antitryptic action of serum constituents, among which are non-saturated fatty acids. The combination of any specific antibody (not

of a ferment nature) with its antigen, *in vitro*, is also capable of removing the antitryptic inhibiting principle from the serum, setting free the protease which, in turn, digests the globulin fraction of the serum and produces dialysable substances.

Incidentally I wish to call attention to the fact that this auto-digestion of serum may explain the mechanism of the phenomenon of the complement-deviation or complement-fixation, for, in each case where complement is fixed, there appear dialysable products that give a positive ninhydrin test and, *vice versa*, wherever the Abderhalden test is positive, the complement (as can be proved) is inactivated.

The auto-digestion of serum, induced by the removal of anti-trypsin in Jobling's experiments, can be stopped by returning non-saturated fatty acid to the serum. The auto-digestion of the serum in the Abderhalden test (which is due to the removal of the anti-tryptic inhibition from the serum of the patient, by the combination of serum antibody with placenta-antigen) can also be stopped by the addition of non-saturated fatty acids. According to my experiments, moreover, self-digestion of the serum results in the production of a toxic substance which appears to be identical with Friedberger's anaphylatoxin and, when occurring *in vivo*, is probably the cause of eclampsia. I am inclined to think from the results of some of our experiments, that here we have the clue to possible prevention of this much dreaded occasional accompaniment of child-birth.

In short, the Abderhalden reaction is specific, but depends not as Abderhalden believes, on the presence of specific enzymes, but on the presence in the blood of pregnant women of the specific antibody that combines with placenta antigen, and thus sets free the only proteolytic enzyme which is always present in the serum of every animal. When considered from this point of view, the Abderhalden test should be positive wherever the complement-deviation test is positive. I have obtained, in many instances, a positive reaction with the sera of syphilitics, using pure lipoid antigen, in which the only source of protein cleavage products was the serum of the patient. This again proves that not the substrate, but the serum itself, is digested in the Abderhalden test.

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A NOTE ON THE ABSENCE OF MORPHINE FROM THE LIVER IN A CASE OF CHRONIC LAUD- ANUM ADDICTION

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There is considerable doubt regarding the nature of the transformations through which morphine may pass after its introduction into the animal body. It is possible that such morphine may be changed into oxidimorphine or some other derivative, or that a compound of morphine with cell material may be formed. However, in many cases of undoubted poisoning by opium or morphine, it has been impossible to detect this drug or alkaloid in the tissues or organs. Witthaus¹ states that Lesser, in a case of post-mortem analysis, found morphine in the urine but not elsewhere in the cadaver. La-saigne² could not find morphine in the liver of a dog poisoned with 8 oz. of Sydenham's laudanum. Christison³ mentions four cases of death due to poisoning from laudanum where no trace of the poison could be detected. Woodman and Tidy⁴ could not detect any alkaloid in a case of laudanum poisoning. Haines⁵ could find no trace of morphine in the stomach in a case where 10 to 15 grains were taken. Haines also quotes the report of Surg.-Maj. Ross, who writes that in Bengal, in 1869, there were 45 fatal cases of poisoning by opium, and an analysis was made in each instance, yet in only two was opium detected in the stomach.⁶

The failures to detect morphine in the urine⁷ in cases of un-

¹ Witthaus and Becker: *Med. Juris., Forens. Med. and Toxicol.*, 1911, iv, p. 977.

² Laissaigne: *Jr. de chim. Med.*, 1841, p. 448.

³ Christison: *On poisons*, 1845, pp. 57, 58, and 537.

⁴ Woodman and Tidy: *Forensic Med. and Toxicol.*, 1877, p. 376.

⁵ Haines: *Hamilton's Legal Med.*, 1894, i, p. 446.

⁶ It is possible that the methods of detection in these cases were faulty.

⁷ Kreyssig: *Dissertation*, Leipzig, 1856; Vogt: *Arch. d. Pharm.*, 1875, vii, p. 23; Landsberg: *Pflüger's Arch.*, 1880, xxiii, p. 413; Burkart: *Weit. Mitth. u. chr. Morph. Vergift.*, Bonn, 1882; Donath: *Pflüger's Arch.*, 1886, xxviii, p. 528; Von Jaksch: *Prag. med. Woch.*, 1897, xxii, p. 477.

doubted opium poisoning, as well as in the urine of morphinists, has also strengthened the idea that the alkaloid is modified or rendered undetectable in the system.⁸ However, Marquis⁹ after the injection of morphine into the circulation of cats, recovered from the liver 30 percent of the injected amount; and Antheaume and Mouneyrat,¹⁰ in a case of morphine poisoning in an individual who had previously used 62 grains daily (recently 30 grains daily), but who had taken no morphine for the preceding two weeks, found morphine in large quantity in the liver.

As morphine, through its phenolic hydroxid, combines with sulfate to form a compound similar in structure to the ethereal sulfate normally contained in urine, the possibility of such a formation in the body suggests itself. Eliassow¹¹ and Stolnikow¹² have shown that the proportion of ethereal sulfates is increased under treatment with morphine.¹³ The results of Rubsamen's¹⁴ experiments tend to show that a certain percentage of injected morphine disappears in the bodies of rats, and that this proportion is increased by habituation. The changes said to occur are effected by oxidation or by "pairing." There has been considerable controversy¹⁵ about these experiments, however, and the matter is still unsettled.

⁸ Cloetta: *Virchow's Arch.*, 1866, xxxv, p. 369; Taylor: *On Poisons*, 3d ed., pp. 556 and 559; Buchner: *N. Rept. f. Pharm.*, 1867, xvi, p. 43; Landsberg: *Pflüger's Arch.*, 1880, xxiii, p. 413; Puschmann: *Dissert.*, Göttingen, 1895; Welmans: *Pharm Ztg.*, 1898, xliii, p. 908; Stursberg: *Arch. Int. de pharmacodyn.*, 1898, iv, p. 333; Bougault: *Compt. rend. Acad. Sci.*, 1902, cxxxiv, p. 1361; Gerard, Delearde et Ricquet: *Jr. de pharm. et de chim.*, 1905, 6S, xxii, p. 49; Stolnikow: *Dissert.*, Lausanne, 1899; Marquis: *Arb. a. d. pharm. Inst. z. Dorpat*, 1896, xiv, p. 118; Strzyzowski: *Dissert.*, Lausanne, 1899.

⁹ Marquis: *Chem. Centralbl.*, 1897, i, p. 249.

¹⁰ Antheaume and Mouneyrat: *Compt. rend.*, 1897, cxxiv, p. 1475.

¹¹ Eliassow: *Dissert.*, Königsberg, 1882.

¹² Stolnikow: *Zeit. f. physiol. Chem.*, 1884, viii, p. 235.

¹³ This might be due, however, to the constipating action of morphine.

¹⁴ Rubsamen: *Arch. f. exp. Path. u. Pharm.*, 1908, lix, p. 227; see also Faust, *ibid.*, 1900, xliv, p. 217.

¹⁵ Marme: *Deut. med. Woch.*, 1883, ix, p. 197; Polstorff: *Berichte*, 1880, xiii, p. 86; 1886, xix, p. 176; Brookmann and Polstorff: *ibid.*, 1880, xiii, p. 88, Pelletier: *Ann. de chim. et de phys.*, 1835, xvi, p. 50; Hesse: *Liebig's Ann.*, 1867, cxli, p. 87; 1875, clxxvi, p. 195; 1883, ccxxii, p. 234; 1886, ccxxxiv, p. 253, ccxxxv, p. 229; Vongerichten: *ibid.*, 1896, ccxciv, p. 206; Lamal: *Bull. Ac. r. de Med. de Belg.*, 1888, 4S, ii, p. 639; *Jr. de pharm. et de chim.*, 1904, xix, p. 61; Diedrich: *Diss.*, Göttingen, 1883; Donath: *J. f. prakt. Chem.*, 1886, xxxiii, p. 559; *Pflüger's Arch.*, 1886, xxxviii, p. 528.

Babel¹⁶ claims that morphine is oxidized by brain pulp *in vitro*, Cloetta¹⁷ previously supposed that nerve tissue is vitally active in this direction. Rubsamen¹⁴ could not verify in rats or rabbits the results of Babel's experiments. Tauber,¹⁸ by perfusion experiments on the liver and kidney of pigs, found that these organs could not oxidize morphine, but Gerard and Ricquet¹⁹ showed that, by maceration with horse kidney pulp, morphine is oxidized to oxidimorphine and the latter is also reduced to the former.

It may be readily noted that there is considerable difference of opinion on the question of the transformation of morphine in the body. I recently obtained the liver, three hours after death, of a woman who had used large amounts of laudanum for about five years. It seemed of interest to determine whether morphine was present in this organ. A careful search for morphine by Dragendorff's process, as described by Witthaus,²⁰ showed that it was absent. As a control, 150 mg. of morphine sulfate were added to a liver; the same amount of morphine sulfate was isolated, proving that the technic was good. This result indicates the possibility that morphine is so changed in the body, that, under conditions as yet unknown, it cannot be recovered.

However, I have shown with Dr. S. R. Mills²¹ that, under certain conditions, morphine withstands decomposition in the presence of putrefying material. Ogier²² states that he has frequently failed to detect morphine in viscera, which had contained it, after putrefaction for from two weeks to one month. Tardieu²³ found morphine in putrefying viscera after 45 days; Nagelvoort²⁴ after 50 days; Marme²⁵ after 8 weeks; Marquis²⁶ after 2 months; Proelss²⁷ after

¹⁶ Babel: *Arch. f. exp. Path. u. Pharm.*, 1905, lii, p. 262.

¹⁷ Cloetta: *Virchow's Arch.*, 1866, xxxv, p. 369.

¹⁸ Tauber: *Arch. f. exp. Path. u. Pharm.*, 1890, xxvii, p. 336.

¹⁹ Gerard and Ricquet: *Compt. rend. soc. biol.*, 1904, lvi, p. 904.

²⁰ Witthaus: *Loc. cit.*

²¹ Rosenbloom and Mills: *Jour. Biol. Chem.*, 1913, xvi, p. 327.

²² Ogier: *Chim. Tox.*, 1899, p. 567.

²³ Tardieu: *Empoisonnement*, 2d ed., p. 1043.

²⁴ Nagelvoort: *Amer. Jr. Pharm.*, 1896, lxviii, p. 374.

²⁵ Marme: *Zeit. f. anal. Chem.*, 1883, xxii, p. 635.

²⁶ Marquis: *Dissert.*, Dorpat, 1896, p. 159.

²⁷ Proelss: *Apoth. Zeit.*, 1901, xvi, p. 492.

260 days; Taylor²⁸ after 14 months; Kauzmann²⁹ after 40 days; Stevenson³⁰ after 60 days; Tidy³¹ after 90 days; Pauzer,³² in two cases, after 6 months; Witthaus,³³ in two cases, after 43 and 53 days, respectively; Autenreith³⁴ after 15 months and Strzyzowski³⁵ after 5 months.

Faust's³⁶ experiments are also of great interest in this connection. He found that, after the hypodermic injection of moderate amounts of morphine into dogs, about 66 percent could be extracted from the feces. By gradually increasing the dose, this amount diminished until, after a time, no morphine was excreted in the urine or feces; and after the death of the animals, none could be extracted from the organs. He thinks that habituation to morphine is due to increased capacity of the tissues to destroy it.

From the results of Autenreith's and Strzyzowski's experiments it appears, however, that morphine undergoes decomposition, which is more extensive with aerobic than with anaerobic putrefaction. Strzyzowski estimates that under certain conditions of putrefaction, 0.5 gm. of morphine mixed with putrefying material would be detectable after 800 days. However it is possible that the effect of the dead cells on morphine is not comparable to the effects of living cells in regard to its oxidation or change into a form or forms that would not be detectable.

The absence of morphine from the liver in the case studied by myself indicates (1) that the morphine was so changed in the organism, under conditions as yet unknown, that it was impossible to detect morphine, and (2) that such a change is marked in cases of habituation to the alkaloid.

²⁸ Taylor: *On Poisons*, 3d ed., p. 556.

²⁹ Kauzmann: *Dragendorff's Beiträge*, p. 131.

³⁰ Stevenson: *Lancet*, 1903, ii, p. 1443.

³¹ Tidy: *Med. Times and Gazette*, 1868, i, p. 497.

³² Pauzer: *Zeit. f. Unt. d. Nahr. u. Genuss.*, 1902, v, p. 8.

³³ Witthaus: *Toxicology*, 1911, p. 982.

³⁴ Autenreith: *Ber. d. deut. pharm. Gesell.*, 1901, xi, p. 494.

³⁵ Strzyzowski: *Dissert.*, Lausanne, 1899.

³⁶ Faust: *Arch. f. exp. Path. u. Pharm.*, 1900, xliv, p. 217.

STUDIES OF SOME COMPOUNDS OF CINCHONA ALKALOIDS, CERTAIN METALS AND PHOSPHORIC ACID*

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During the year 1900, under the direction of Prof. J. W. Mallet of the University of Virginia, I undertook some studies of compounds of alkaloids and metals. The alkaloids were principally those of cinchona, and the metals were of several groups. This work was abandoned before anything definite was accomplished, although I had reason to believe that some compounds had been made. In an attempt to obtain better means of treating gonorrhoeal urethritis than was available, I again turned my attention, in 1910, to a study of alkaloidal and metallic compounds.

Cinchona alkaloids, especially quinin, were studied because of their protoplasmic poisonous qualities, and the fact that there had been some success with quinin in the treatment of infections. The success of Helmholtz and others with quinin as an antiseptic warranted a close study of it. The known gonococidal effect of silver commended that metal.

Many efforts to combine different acids and various radicals with quinin and silver resulted in failure until orthophosphoric acid was tried. An aqueous sol. of silver nitrate was treated with a conc. sol. of sodium phosphate to complete precipitation of the silver as phosphate. The yellow silver phosphate was washed by decantation and then on a filter. It was then treated with syrupy orthophosphoric acid to complete solution. The resulting sol. was treated with pure quinin until no more of the alkaloid was taken up. As the point of saturation was reached, the sol. changed to a darker color.

This solution was used clinically, diluted as found best by trial. There is no intention of going into a clinical discussion in this com-

* Proceedings of the Columbia University Biochemical Association, Feb. 5, 1915; *BIOCHEM. BULL.*, 1915, iv, p. 227.

munication. Suffice it to say that the results from the use of the sol. in the treatment of gonorrhœa have been most gratifying to those who have used it. My colleagues here and in other places have reported to me splendid success with it. Extending its use I tried it on chancroids, tonsillitis, ulcers of various kinds, and in one case of amebic infection of the colon. This last case was reported in the *Journal of the American Medical Association*, vol. lx, pp. 1357 and 1358 (1913). It has been found especially beneficial in chronic gonorrhœal urethritis and in gonorrhœa in women.

Until recently I had no positive evidence that I had made a *compound* of silver, quinin and the acid. All attempts to obtain crystals met with failure. Almost by accident a crystal was found in a conc. sol. which had stood unmolested in a dark cabinet from October, 1913, to December, 1914. On closer investigation two complex crystals were found in the bottom of the flask containing the conc. sol. which had stood 15 months. These were removed, carefully washed and dried. They were very dense; their color was dark yellow. One of them was used in demonstrating the presence of silver, quinin and phosphoric acid; the other is now in safe keeping for further investigation.

The crystal was decomposed in heated strong nitric acid, and the presence of silver demonstrated by precipitation with sodium chlorid and the character of the resulting precipitate. An ammonium phosphomolybdate precipitate was then obtained. On addition of strong ammonia to the nitric acid sol. of the crystal, a heavy yellow precipitate was thrown down, which was dissolved with excess of ammonia when, in the top of the sol., there appeared a flocculent white precipitate that proved to be quinin. One of my associates went over the work with me, so that there could be no mistake in it. A quantitative analysis of the crystal has not been made. That will be done at an early opportunity.

In place of silver I have made sol. of copper phosphate and zinc phosphate with quinin, quininidin, cinchonin and cinchonidin. The relative merits clinically of these sol. remains for future determination. No crystals of these compounds, if they be such, have been obtained.

ON THE ACCELERATION OF THE OXIDATION OF ALUMINIUM BY MEANS OF MERCURY

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Our knowledge of oxidations in the body is so meagre that any observations on rapid oxidations at room temperatures outside the body may be of interest. Although many accelerators (enzymes) have been extracted from living cells, such extracts, after being centrifuged, are incapable of oxidizing any of the ordinary food stuffs to carbon dioxid and water. With the aid of adsorption surfaces, carbon dioxid may be produced by some tissue extracts, but the complicated relations involved are very difficult to investigate. Unsaturated fatty acids and their compounds (such as lecithin) oxidize spontaneously in the air but no carbon dioxid is produced. Oxalic acid is completely oxidized by blood charcoal and oxygen in water; but in this case one active oxygen atom is sufficient to oxidize a whole molecule of the acid, or the molecule of formic acid, if it is split into carbon dioxid and formic acid. A less complete oxidation would hardly be expected.

A number of inorganic accelerators have been found and I wish to add one to the list. If a trace of mercury is driven into a piece of aluminium by means of an electric spark, the aluminium will burn in dry air (humidity 10 percent at 20° C.) at a rapid rate. A voluminous oxid is formed so fast that its increase may be easily detected by continuous observation for a few seconds with the naked eye or a low-power lens. The masses of white oxid grow out of the metal as plants grow out of the ground, attaining the height of a millimeter in a few minutes. In this process, the energy liberated by oxidation is partly expended in lifting the weight of the oxid against gravity, in the same way that part of the energy of oxidations in the body is ultimately expended in lifting the body during growth.

THE DETOXICATING EFFECT OF THE LIVER OF CATHARTES AURA UPON SOLUTIONS OF β -IMIDAZOLYLETHYLAMIN*

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This research was undertaken with an idea of explaining some of the clinical phenomena observed in cases of intestinal toxemia. Many cases are observed by clinicians in which there is very marked indicanuria, but in which there are no subjective symptoms; while other cases may present decided subjective symptoms with only moderate indicanuria.

Most physiologists overlook a very important function of the liver which, to the writer, appears to be its chief function so far as prolongation of life is concerned. We know how soon an animal may die after the institution of an Eck fistula, and yet we meet cases in which the glycogenic (diabetes) and biliary functions (biliary cirrhosis) of this organ are greatly disturbed, or altogether lacking, with little impairment of health for a long time.

The presence of indican in the urine is an example of the results of the detoxicating action of the liver cells upon an intestinal toxin. That such action obtains in the case of other intestinal poisons is shown by the experiments of Ewins and Laidlaw (1) who have shown that *p*-oxyphenylethylamin, when perfused through the liver of a cat, is broken up into *p*-oxyphenylacetic acid and urea, which are non-toxic.

The liver of the common turkey buzzard, *Cathartes aura*, was chosen for the following experiments on account of its well-known fondness for carrion, upon which it apparently thrives. An adult bird, after having been trapped and kept in a cage for 3 days on a diet of fresh raw meat, was killed by a rifle bullet through its head. It was then immediately skinned, care having been taken to avoid

* Proceedings of the Columbia University Biochemical Association, Feb. 5, 1915; *BIOCHEM. BULL.*, 1915, iv, p. 224.

opening of the peritoneal cavity. With the carcass lying on its back, the muscles and fascia to the right of the median line of the abdomen were thoroughly cooked with a soldering iron. An incision was made, with a sterile scalpel, through the cooked tissues into the peritoneal cavity. The liver was removed piecemeal, with sterile scissors and forceps, and placed in a sterile mortar with sterile broken glass, and then ground to a pulp. No effort was made to weigh the liver-substance used, all endeavors aiming at transference to sterile flasks as soon as possible, to avoid contamination. Approximately 10 gm. of the liver pulp and glass were placed in one Erlenmeyer flask (a) and about 20 gm. in another (b), while the third flask was a control (c):

Liver pulp	A, 10 gm.	B, 20 gm.	C, none
β -Imidazolylethylamin in saline sol., 1-1000 (c.c.)...	10 c.c.	15 c.c.	5 c.c.
Toluene (c.c.)	4	4	4

All flasks were incubated at 37° C., for 24 hr. Inoculations from all flasks were then made on agar and in bouillon to test the sterility, which showed no growth in 48 hr. The contents of the flasks were filtered and the filtrates used for injections into guinea-pigs. Dale (2), as well as the writer (3), has shown that 0.5 mg. of β -imidazolylethylamin, injected into the blood stream, kills a 300 gm. guinea-pig in 6 min., from spasm of the bronchioles and suffocation. Injected into guinea-pigs on a basis of 0.5 mg. per 300 gm. of weight, there was no effect for the solutions that were incubated with liver, but for the control solution there were the usual fatal symptoms.

That this action is due to some enzyme seems probable, for heating to boiling inhibits the detoxicating action. Further study will no doubt elucidate this problem but the scarcity of material has, for the present, required postponement of the experiments. It is also of interest to note that the power of causing urticarial lesions, possessed by this amin, to which the writer called attention last year (4), is also destroyed by heat.

Full protocols of these experiments will be published as soon as a sufficient quantity of the amin can be obtained for final tests, but justification for this preliminary note is found in the hope that some one more fortunate than the writer may have sufficient of the amin to be able to complete the study; or that these results may lead

to further experimental work on the several amines of intestinal origin, with a view to extending our knowledge on the detoxicating function of the liver.

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THE ORGANIC PHOSPHORUS COMPOUNDS OF WHEAT-BRAN

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Introduction. The organic-phosphorus materials, or phytins, obtained by alcoholic precipitation of aqueous or dilute acid extracts from various sources, are not identical, but ultimate analyses show a fair degree of similarity. Thus, the phosphorus content varies between 14 and 17 percent, and there are varying proportions of magnesium, potassium and calcium. There has been prepared, also, from the phytins from many sources, the free phytic acid, corresponding to the formula $C_2H_8P_2O_9$ (anhydro-oxy-methylene phosphoric acid, Posternak),¹ or $C_6H_{24}P_6O_{27}$ (Neuberg,² Starkenstein³).

In the case of the material extracted from wheat bran, however, there has been difference of opinion regarding its identity with phytin and its ability to yield phytic acid. Patten and Hart⁴ claimed to have obtained an acid containing 10.63 percent of carbon, 3.38 percent of hydrogen, and 25.98 percent of phosphorus, figures agreeing very well with the formula $C_6H_{24}O_{27}P_6$. They therefore called their product phytic acid. Anderson,⁵ on the other hand, was unable to obtain such a compound, and ascribed Patten and Hart's supposed error to contamination with inorganic phosphates and phosphoric acid. Anderson obtained his material by a method of procedure different from that used by Patten and Hart, a fact that may explain the divergent results.

It was with a view to clearing up this matter that the work described in this paper was undertaken. Wheat-bran contains a

¹ Posternak: *Rev. gen. de bot.*, 1900, xii, pp. 5 and 65.

² Neuberg: *Biochem. Zeitschr.*, 1908, ix, pp. 551 and 557.

³ Starkenstein: *Ibid.*, 1911, xxx, p. 56.

⁴ Patten and Hart: *Compt. rend. de l'acad. des sci.*, 1903, cxxxvii, Nos. 3, 5 and 8.

⁵ Anderson: *Jour. Biol. Chem.*, 1912, xii, p. 447.

much larger percentage of organic-phosphorus extractives than most other materials so far examined, and probably is the best source of phytin and phytic acid for further investigations.

We have repeated Patten and Hart's work. Their so-called tri-barium phytate has been prepared from wheat bran, with care to insure the absence of inorganic phosphates by means of the method recommended by Anderson, viz., repeated solution of the salt in dilute hydrochloric acid sol. and reprecipitation with alcohol. With Anderson's new method,⁶ we have been able to prepare this barium salt in crystalline form and identical in properties with that obtained by him from cotton-seed meal, oats and corn, but corresponding more closely in composition with the formula, $C_6H_{18}O_{24}P_6Ba_3$, than with Anderson's formula $C_6H_{12}O_{24}P_6Ba_3$. Our data leave no question as to the presence of substances in wheat-bran which yield, by the usual treatment to be described in the experimental part, a substance very similar to phytic acid, but apparently having the composition represented by the formula $C_6H_{24}O_{24}P_6$. From his crystalline tri-barium salt from cotton-seed meal, oats and corn, Anderson obtained an acid to which he ascribed the formula, $C_6H_{18}O_{24}P_6$. Hence, both in the case of the barium salt and the free acid, our compounds appear to contain six more hydrogen atoms to the molecule; while in carbon, barium and phosphorus contents, they agree very well with Anderson's compounds.

In comparing the results of the analyses, the method used in combustion must be taken into consideration. It is a well known fact that, in the combustion of organic compounds containing phosphorus, the phosphorus is converted into metaphosphoric acid, HPO_3 , which remains as a glossy coating in the boat, and may occlude more or less carbon. Anderson states that in decomposing his crystalline barium salts, it was necessary to burn a second time with chromic acid, in order to insure combustion of all the carbon, but that this was unnecessary with the amorphous barium salts. Since he does not say that he burned the free acid with chromic acid, we presume he did not do so. It is inevitable, if this is true, that his hydrogen analyses gave low results for phytic acid. It is a noteworthy fact that his formula shows six atoms less in the

⁶ Anderson: *Jour. Biol. Chem.*, 1914, xvii, p. 160.

molecule than ours; and, since the molecule contains six atoms of phosphorus, the formation of the metaphosphoric acid residue would account for the discrepancy, if our substance is identical with his. In the case of the barium salt, the explanation is less evident, for, of course, barium phosphate or metaphosphate would be formed, together with some barium carbonate and metaphosphoric acid, although a reaction between the latter two substances might take place, liberating both the hydrogen and carbon.

In each of our combustions, we burned the material a second time: in the case of the free acid and the brucine salt to be described, with well dried, powdered lead chromate; in the case of the barium salts, with a mixture of lead chromate and potassium dichromate. There was always an increase in weight in both the potash bulbs and the calcium chloride tube after the second burning. It is probable, therefore, that our compounds from wheat-bran are identical with those obtained by Anderson from various other sources.

We believe, however, that in addition to the phytic acid derivative in our extracts of wheat-bran, there were at least two other organic-phosphorus compounds, which we have been prevented from investigating completely by lack of time. It was one of these substances which Anderson⁷ investigated, and found to yield an acid, to which he ascribed the formula, $C_{20}H_{55}O_{49}P_9$, combined with the elements of a pentose. In regard to this substance, we wish to point out that his analytical results show rather wide departures from the calculated formula, and that none of the barium salts were obtained crystalline; hence may not have been pure. It is also noteworthy that the analytic data for the crystalline brucine salt [to which he ascribed the formula $C_{20}H_{55}O_{49}P_9 \cdot (C_{23}H_{26}O_4N_2)_{10}$], accorded better (except in the case of carbon which is low) with brucine phosphate, $(C_{23}H_{26}O_4N_2)_3 \cdot (H_3PO_4)_2$ than with his calculated formula. Anderson⁸ himself has shown that phytic acid is broken down into phosphoric acid and other substances by drying at 100° C., and even to some extent by drying at ordinary temperatures. The new acid prepared by him from wheat-bran

⁷ Anderson: *Jour. Biol. Chem.*, 1912, xii, p. 450.

⁸ Anderson: *Ibid.*, 1914, xvii, p. 171.

was found to yield inosite and phosphoric acid on hydrolysis with acid, and hence possibly also on drying. At any rate, we have been unable to obtain a crystalline salt of brucine by using a preparation which had not first been heated. After drying about 1 gm. of the acid at 100° C. for several hours, however, we obtained a good yield of crystals, corresponding in physical properties with, and approximating in composition, Anderson's brucine salt; and also with pure brucine phosphate, prepared and analyzed by us.

The description of the experimental work is divided into three parts. The first part deals with an investigation of a precipitate obtained by adding copper acetate sol. to an extract of wheat-bran. The second part relates to the material resulting from alcoholic precipitation of bran extract. The third part describes a combination of the two methods.

Experimental part. 1. PRECIPITATE OBTAINED WITH COPPER ACETATE. *Preparation of the impure barium salt.* Five kilos of wheat-bran were extracted over night in 30 l. of 0.2 percent hydrochloric acid sol., the liquid then strained and pressed out of the residue, and 16 l. more of the 0.2 percent acid sol. added. After stirring at intervals for 2 hr., this was strained out, and the two extracts united. After standing for some time, to allow suspended matter to settle out, the supernatant liquid was strained through cotton. To the filtrate was added an excess of conc. sol. of copper acetate, containing some acetic acid. A heavy precipitate was produced. This was allowed to settle over night, the precipitate filtered on a Buchner funnel, and washed two or three times with water. It was then suspended in water, and hydrogen sulfid gas run in for several hours, the mixture being stirred constantly by means of a water motor. The liquid was then filtered from the precipitated copper sulfid. To the filtrate was added a sol. of 100 gm. of barium chlorid, and then barium hydroxid sol. to strong alkaline reaction. A heavy precipitate was obtained. This was filtered out, dissolved in dil. hydrochloric acid sol. and filtered from a slight insoluble residue. To the filtrate was again added some barium chlorid and barium hydroxid sol. to alkaline reaction. After filtering and dissolving the precipitate in dilute hydrochloric acid sol., it was precipitated with 3 vol. of alcohol. The precipitate,

after resolution in dil. hydrochloric acid sol., was again precipitated with alcohol. This process was repeated three times more. The material was now free from inorganic phosphates, *i. e.*, it gave no yellow precipitate when warmed with molybdc sol. After washing in alcohol and ether, and drying, the product weighed 57 gm. Dried at 130° C. for analysis, this material turned slightly gray. Analytic data:

	0.2080 gm. gave 0.1235 gm. $Mg_2P_2O_7$.
	0.4736 gm. gave 0.2853 gm. $BaSO_4$.
	1.0058 gm. gave 0.00098 gm. N, by Kjeldahl method.
<i>Found:</i>	P, 16.55%; Ba, 35.54%; N, 0.098%.
<i>Calculated:</i>	for tri-barium inosite-hexaphosphate, $C_6H_{12}O_{24}P_6Ba_3$ —
	P, 17.44%; Ba, 38.65%.

In elementary composition this material approaches the constitution of a phytin derivative more closely than does Anderson's product, but it is low in both phosphorus and barium. Believing it to be a mixture of tri-barium phytate and some other substance, a means of effecting a separation was sought.

Separation by dialysis. One gm. of the material, dried at 100° C., was dissolved in dil. hydrochloric acid sol., and placed in a S. & S. No. 579, dialyzing capsule, the latter being put into a beaker of distilled water. After 48 hr., the dialysate and the material remaining in the capsule were precipitated with 3 vol. of alcohol. After filtering out both precipitates, and washing with alcohol and ether, and drying, it was found that the undialyzable material weighed 0.2083 gm. Although precipitation was evidently incomplete, it was plain that some, at least, of the substance was capable of dialysis. Barium was determined in both fractions:

	0.2083 gm. gave 0.1213 gm. $BaSO_4$ (undialyzed fraction).
	0.3820 gm. gave 0.2408 gm. $BaSO_4$ (dialysate).
<i>Found:</i>	in the dialysate, 37.09 per cent. Ba; in the non-dialyzable fraction, 34.27 per cent. Ba.

From these data it is evident that there are at least two substances in the crude barium-product obtained from the wheat bran, and that the one having the higher percentage of barium is dialyzable. Since no attempt was made to effect complete separation, by changing the water in the outer container, the undialyzed material

was, of course, contaminated by some of the dialyzable portion, so that the true barium content must be lower.

Separation by extraction with water. This method was suggested and used by Anderson⁹ in purifying the phytin derivative from oats. Ten gm. of crude barium preparation were rubbed up in a mortar with about 50 c.c. of water and, after standing for a time, filtered. The residue was extracted twice more in this way with small quantities of water, and finally washed with water, alcohol and ether, and dried. The aqueous extract was slightly yellow. Addition of alcohol produced, in the first two portions of extract, a rather abundant precipitate; but in the third portion, only a faint turbidity, showing that the extraction was fairly complete. The precipitate, after being filtered out and dried, weighed 1.1 gm. The water-insoluble material was pure white, while the water-soluble fraction was slightly yellow. Analytic data:

WATER-INSOLUBLE FRACTION.

Dried for analysis at 100° C.

0.4972 gm. gave 0.3115 gm. BaSO₄.

0.3210 gm. gave 0.0650 gm. CO₂ and 0.0516 gm. H₂O.

0.2946 gm. gave 0.1676 gm. Mg₂P₂O₇.

Found: Ba, 36.87%; P, 15.86%; C, 5.52%; H, 1.80%.

Calculated: for tri-barium inosite-hexaphosphate, C₆H₁₂O₂₄P₆Ba₃—

Ba, 38.65%; P, 17.44%; C, 6.75%; H, 1.12%.

WATER-SOLUBLE FRACTION.

Dried for analysis at 100° C.

0.4448 gm. gave 0.2560 gm. BaSO₄.

0.2300 gm. gave 0.0630 gm. CO₂ and 0.0410 gm. H₂O.

Found: Ba, 33.87%; C, 7.47%; H, 2.00%.

This substance has not been investigated further.

Preparation of crystalline barium salt. Five gm. of the water-insoluble fraction were dissolved in the smallest possible quantity of dil. hydrochloric acid sol., a sol. of pure barium hydroxid was added to nearly neutralize the free acid, together with 10 gm. of barium chlorid in conc. sol. The mixture was filtered, and alcohol added until a slight permanent precipitate resulted. This precipitate was amorphous. After standing over night, it was crystalline or pseudo-crystalline, the material having aggregated in the form

⁹ Anderson: *Jour. Biol. Chem.*, 1914, xvii, p. 160.

of microscopic globules, similar to those described by Anderson from cotton-seed meal, oats and corn. After filtration, second, third and fourth crops of these crystals, as we shall call them, were obtained by adding more alcohol and allowing to stand. These were united, and recrystallized by the same procedure. About 2 gm. of pure white substance were thus obtained. The crystals were dried for analysis at 105° C., in vacuum over phosphorus pentoxid. Analytic data:

0.2124 gm. gave 0.1274 gm. $Mg_2P_2O_7$.

0.2053 gm. gave 0.1347 gm. $BaSO_4$.

0.3099 gm. gave 0.0708 gm. CO_2 and 0.0392 gm. H_2O .

Found: P, 16.72%; Ba, 38.61%; C, 6.23%; H, 1.42%.

Calculated: for tri-barium inosite-hexaphosphate, $C_6H_{12}O_{24}P_6Ba_3$ —

P, 17.44%; Ba, 38.65%; C, 6.75%; H, 1.12%.

Calculated: for $C_6H_{18}O_{24}P_6Ba_3$ —

P, 17.35%; Ba, 38.43%; C, 6.72%; H, 1.69%.

There is little choice between these two calculated formulas. While the results for this material correspond to those for a tri-barium salt, the crystalline substance obtained by Anderson (by the same method) gave analytic data corresponding to the heptabarium salt, (R_2Ba_7) . It is possible that our solution contained more free acid than his. When portions of this salt that had been dried in vacuum over sulfuric acid at room temperature, or in a water oven at 100° C., were further dried at 105° C., in vacuum over phosphorus pentoxid, a slight loss in weight resulted. This was, however, variable; the crystal form of the salt was not injured by it. It hardly seems probable, therefore, that water of crystallization was present, hence the percentages of moisture lost by this drying are not quoted.

Crystallization of the barium salt from dilute acid solution. All of the remaining impure barium salt was extracted with water as described above, and the insoluble portion, weighing about 20 gm., purified as follows. It was dissolved in 0.2 percent hydrochloric acid sol. and, after filtering from a slight insoluble residue, alcohol was added until a fairly heavy precipitate resulted. This required considerably less than 1 vol. of alcohol. The precipitate was amorphous but, after standing over night, it became crystalline, similar in appearance to that already described. It was filtered

out, washed with alcohol and ether, and dried. After securing second and third crops of crystals, all were united and recrystallized in the same way. After drying in a water-oven for some time at 100° C., the product, weighing about 7 gm., was a light, powdery material. Part of this was dried at 105° C., in vacuum over phosphorus pentoxid, and analyzed. Analytic data:

0.1400 gm. gave 0.0860 gm. $Mg_2P_2O_7$.

0.1596 gm. gave 0.1001 gm. $BaSO_4$.

0.2275 gm. gave 0.0575 gm. CO_2 and 0.0399 gm. H_2O .

Found: P, 17.12%; Ba, 37.77%; C, 6.89%; H, 1.96%.

Calculated: for $C_6H_{18}O_{24}P_6Ba_3$ —

P, 17.35%; Ba, 38.43%; C, 6.72%; H, 1.69%.

Preparation of the free acid from the crystallized material. The entire amount of the crystalline material remaining, a little less than 7 gm., was decomposed as follows. Somewhat more than the calculated amount of normal sulfuric acid sol. was added to precipitate the barium and, after warming for some time, the liquid was filtered. To this was added an excess of copper acetate sol., and the precipitate filtered out and washed thoroughly with water. It was finally suspended in water, and decomposed with hydrogen sulfid gas. After filtering from the copper sulfid, the liquid containing phytic acid was concentrated to a small bulk by boiling in vacuum, the temperature not rising above 65° C. The residue was finally dried for ten days in vacuum over sulfuric acid at room temperature. The residue, weighing about 3 gm., was a very thick, amber colored syrup. For analysis a portion of it was dried at 105° C. in vacuum over phosphorus pentoxid. Analytic data:

0.1208 gm. gave 0.1199 gm. $Mg_2P_2O_7$.

0.2893 gm. gave 0.1156 gm. CO_2 and 0.0917 gm. H_2O .

Found: P, 27.67%; C, 10.90%; H, 3.54%.

Calculated: for $C_6H_{24}O_{24}P_6$ —

P, 27.92%; C, 10.81%; H, 3.63%.

Calculated: for $C_6H_{18}O_{24}P_6$ —

P, 28.18%; C, 10.90%; H, 2.73%.

Drying at 105° C. caused blackening, and, presumably, partial decomposition of the material. Anderson¹⁰ shows, in the case of

¹⁰ Anderson: *Jour. Biol. Chem.*, 1914, xvii, p. 171.

the similar acid from cotton-seed meal, corn and oats, that such drying causes the formation of phosphoric acid. We have found that this is true for our product from wheat-bran. Analytic data:

0.1919 gm. unheated acid gave 0.0070 gm. $Mg_2P_2O_7$.

0.2360 gm. acid heated to $105^\circ C.$ gave 0.0381 gm. $Mg_2P_2O_7$.

0.2570 gm. unheated acid gave, after heating at $105^\circ C.$, 0.0210 gm. H_2O .

The unheated acid therefore contains 8.17 percent of water. Stating the results on the dry basis: before heating, 4.00 percent of the total phosphorus was present as phosphoric acid, a part of which may have been produced by the nitric acid of the molybdate solution. After heating for 2 hr. at $105^\circ C.$, 16.26 percent of the total phosphorus was present as phosphoric acid.

Attempt to prepare brucine phytate. Hoping that it might be possible to prepare a crystalline brucine salt of phytic acid, that could be compared with the brucine salt prepared by Anderson from his more complex acid, we undertook to make it by the method used by him, except that to begin with, we did not use the dried acid. This work was done before we effected a separation of the crude barium salt by water extraction, and the mixture of the water soluble and insoluble materials was therefore used. To 10 gm. of this material, the calculated amount of normal sulfuric acid sol. was added to precipitate the barium. After filtering, the filtrate was concentrated, by boiling in vacuum, to a small bulk. An excess of crystallized brucine was added and, in turn, 150 c.c. of alcohol, 15 c.c. of chloroform, and ether until a permanent turbidity resulted. After standing for two weeks with occasional addition of ether, at a temperature most of the time below freezing, there was not a trace of crystalline deposit, although there was a small amount of gummy material on the bottom of the flask.

To 3 gm. of the impure barium salt was added the calculated amount of normal sulfuric acid sol., and the liquid, after filtering, evaporated on a water bath, and dried in a water oven for 24 hr. The black residue was dissolved in a small amount of water, alcohol added and the solution filtered from a small amount of insoluble, carbonaceous matter. Brucine, chloroform and ether were then added as before and, after standing for 1 hr. in the laboratory, there

began to form a deposit of fine needles. After standing in the cold for several days, these were filtered out, and recrystallized by the same procedure. About 0.8 gm. of crystals were obtained. These were soluble in water and alcohol, but insoluble in ether and chloroform. No sharp melting point could be obtained, the substance gradually melting with decomposition between 187° C. and 200° C. The remaining material was dried for analysis at 100° C. Analytic data:

0.3207 gm. gave, by Kjeldahl method, NH_3 to neutralize 12.84 c.c. $n/10$ H_2SO_4 .

0.2916 gm. gave 0.0684 gm. $\text{Mg}_2\text{P}_2\text{O}_7$.

0.1087 gm. gave 0.2224 gm. CO_2 and 0.0624 gm. H_2O .

Found: C, 55.80%; H, 6.42%; P, 5.55%; N, 5.61%.

Found: by Anderson from the other acid, $(\text{C}_{20}\text{H}_{35}\text{O}_4\text{P})_2$ —

C, 56.24%; H, 6.26%; P, 4.69%; N, 5.88%.

Calculated: for brucine phosphate, $(\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_4)_3(\text{H}_3\text{PO}_4)_2$ —

C, 59.53%; H, 6.08%; P, 4.46%; N, 6.04%.

Obtaining thus a crystalline compound agreeing closely, both in properties and composition, with that obtained by Anderson from a different acid, we can account for the result only by supposing that the acids were decomposed by heat into some other material, common to both—which could be only phosphoric acid.

Preparation of brucine phosphate. An unweighed amount of syrupy phosphoric acid was diluted somewhat with water, brucine added, then alcohol, chloroform and ether. Almost immediately a deposit of fine needles appeared, which increased in amount on standing over night. These were filtered out, recrystallized as before, and dried for analysis at 100° C. Their physical properties were identical with the material previously prepared. Analytic data:

0.5658 gm. gave 0.0872 gm. $\text{Mg}_2\text{P}_2\text{O}_7$.

0.3400 gm. gave, by Kjeldahl method, NH_3 to neutralize 14.97 c.c. $n/10$ H_2SO_4 .

0.4198 gm. gave, by Kjeldahl method, NH_3 to neutralize 18.25 c.c. $n/10$ H_2SO_4 .

0.1550 gm. gave 0.3301 gm. CO_2 and 0.0891 gm. H_2O .

Found:

C, 59.66%; H, 6.43%; P, 4.53%; N, 6.18% and 5.85%.

Calculated: for brucine phosphate, $(\text{C}_{23}\text{H}_{26}\text{N}_2\text{O}_4)_3(\text{H}_3\text{PO}_4)_2$ —

C, 59.53%; H, 6.08%; P, 4.46%; N, 6.04%.

It seems probable, therefore, that the substances obtained by Anderson and by us, from the organic-phosphorus acids, is nothing

but impure brucine phosphate, the phosphoric acid being produced by hydrolysis.

Attempt to prepare the ethyl ester of phytic acid. An attempt was made to prepare the ethyl ester of this organo-phosphoric acid, by treating the silver salt, prepared from silver oxid, with ethyl iodid; but only an impure product, containing free iodine, was obtained. This impure product was soluble in nitrobenzene.

2. PRECIPITATE OBTAINED BY MEANS OF ALCOHOL. The work represented in this part of the paper is of interest chiefly by comparison with the results of part III. Wheat-bran extract was treated by the method used by Anderson for the preparation of the compound $C_{25}H_{55}O_{59}P_9Ba_5$, and we obtained a substance giving an analysis fairly close to that obtained by him.

Preparation of the crude phosphorus compound by Anderson's method. Five kilos of wheat-bran were extracted over night in 0.2 percent hydrochloric acid sol. To the extract, after straining through cloth, was added dry tannic acid to precipitate the protein material. A very heavy purplish precipitate was produced. This was filtered out, and to the filtrate was added one vol. of alcohol. A heavy white precipitate appeared at once. This was allowed to settle, the liquid siphoned off, and the precipitate collected on a large Buchner funnel, without suction (the method which was found to be most satisfactory in working with all these compounds). The precipitate was redissolved in dil. hydrochloric acid sol., the solution obtained being milky and filtering only with difficulty. In attempting to overcome this trouble, more tannic acid was added, producing a precipitate which soon became gummy, and from which a perfectly clear filtrate could be obtained. One vol. of alcohol was added, and the resulting precipitate was purified by dissolving in dil. acid sol. and precipitating with alcohol, repeating five times. The precipitate, instead of being light and flocculent, was rather heavy, and soon settled into a gummy mass, which could be removed from the solution with a glass rod. In the last precipitation, it was found that 3 vol. of alcohol were necessary to throw down the substance completely, so that considerable of the material was lost. This statement may have some bearing on the results in the third part of this paper. The gummy substance was dried in a vacuum

desiccator, a white, opaque solid being produced, weighing 10 gm. This could be readily powdered in a mortar. It gave a strong acid reaction to litmus paper. The substance was dried for analysis at 100° C. Analytic data:

0.3052 gm. gave 0.1563 gm. $Mg_2P_2O_7$.

0.2163 gm. gave 0.1385 gm. CO_2 and 0.0828 gm. H_2O .

Found: P, 14.27%; C, 17.46%; H, 4.32%.

Quantitative determinations of nitrogen were not made, but qualitative tests showed its presence in traces only. Qualitative tests for bases showed magnesium and potassium in fairly large quantities, calcium and sodium in traces.

This material, after treatment with boiling hydrochloric acid sol., reduced Fehling sol. but not before. The vapors from the boiling mixture colored a strip of anilin-acetate paper pink, indicating the production of furfural from pentose. Polariscopic examination of a 10 percent sol. showed optical inactivity. This solution was boiled for some time with an equal vol. of conc. hydrochloric acid sol., the water lost by evaporation being replaced, and the solution, which had darkened considerably, was decolorized with animal charcoal. Polariscopic examination now showed what was judged to be a very slight dextro-rotation, but so slight as to be uncertain.

Preparation of the barium salt. All the remaining material, weighing 5.5 gm., was dissolved in 200 c.c. of dil. hydrochloric acid sol. and, after heating nearly to boiling, barium hydroxid sol. was added to alkalin reaction and the precipitate filtered out. The filtrate was reserved for further examination. The precipitate was dissolved in dil. hydrochloric acid sol. and barium hydroxid again added to alkalin reaction. The precipitate was again dissolved in dil. acid sol. and reprecipitated with 3 vol. of alcohol. After undergoing three more purifications by precipitation with alcohol, the substance was washed with alcohol and ether, and dried. The product, weighing 2.5 gm., was a white amorphous powder, having an acid reaction. Dried for analysis at 130° C., the material turned slightly gray. Analytic data:

0.3017 gm. gave 0.1568 gm. $BaSO_4$ and 0.1430 gm. $Mg_2P_2O_7$.

Found: Ba, 30.59%; P, 13.16%.

Found: by Anderson— Ba, 31.29%; P, 12.71%.

We did not make carbon and hydrogen analyses of this material. So far as examined, however, this substance appeared very similar to that prepared by Anderson.

Examination of filtrate from barium precipitation of phytin solution. This filtrate was freed from barium with carbon dioxide, filtered and concentrated on a water bath to a small volume, and again filtered from traces of barium carbonate. The residue, after further concentration, was a yellowish, somewhat viscous liquid, with a very peculiar odor, somewhat like old, but not putrid, egg-yolk. The taste was not marked. It reduced Fehling sol. on boiling, but did not give the anilin-acetate test for furfural on boiling with hydrochloric acid. It gave heavy precipitates with phosphotungstic acid, picric acid and tannic acid. Phosphorus was present, as was also nitrogen. Dried in vacuum, the material seemed somewhat crystalline, but was sticky and hygroscopic.

3. PRECIPITATES OBTAINED BY A COMBINATION OF TREATMENTS WITH COPPER ACETATE (1) AND ALCOHOL (2). From the fact that the two different methods used in the first and second parts of this paper yielded different products, it appeared possible that neither method alone was sufficient to secure a complete removal of all the organic-phosphorus compounds in the wheat-bran extract. Supposing this to be true, it should be possible by combining the two methods, to obtain two fractions of precipitate, thus not only securing a more nearly complete precipitation, but also establishing the presence of two different substances in the extract. By precipitating the acid extract first with 3 vol. of alcohol, removing this precipitate, and treating the filtrate with copper-acetate sol., we hoped to effect this separation. The results obtained are somewhat surprising in the light of the data in the first two parts of this paper, and we are unable at this time to offer an adequate explanation for them. The precipitate produced by alcohol, upon purification and formation of the barium salt, yielded, instead of the 31 percent barium salt of part 2, prepared by the same method, the 36 percent barium salt of part 1, prepared by the copper acetate method, and like it, separable into water-soluble and water-insoluble materials, the latter obtainable only in an impure form, but semi-crystallizable. The copper acetate product from the alcoholic

filtrate gave a heavy precipitate, consisting almost entirely of inorganic phosphate, since, after being converted to the barium salt, it failed to precipitate with alcohol.

Preparation of the alcoholic precipitate (2). Two and one-half k. of wheat-bran were extracted as before with 0.2 percent hydrochloric acid sol. over night, and the extract, after filtering, precipitated directly with 3 vol. of alcohol, without previous purification with tannic acid, which would have interfered with the copper precipitation of the filtrate. The precipitate, after settling, was filtered out, dissolved in 0.2 percent hydrochloric acid sol., and tannic acid added in excess. The resulting precipitate was filtered out, and the filtrate precipitated with alcohol. The precipitate was then purified by four more alcoholic precipitations, and was finally washed with alcohol and ether, and dried. Yield: 40 gm.; free from inorganic phosphates and slowly but perfectly soluble in water.

Copper acetate precipitation of filtrate (1). To the alcoholic filtrate was added a conc. sol. of 100 gm. of copper acetate. A very heavy precipitate was produced. This was filtered and washed, suspended in water, and decomposed with hydrogen sulfid. The filtrate from the copper sulfid was made alkaline with barium hydroxid sol., after the addition of a sol. of 100 gm. of barium chlorid, a heavy white precipitate resulting. This was filtered out, dissolved in 0.2 percent hydrochloric acid sol., and 3 vol. of alcohol added. Only a faint turbidity was produced, and after long standing a slight precipitate formed which, after filtering and drying without further purification, weighed only about 0.2 gm. It was discarded. It is possible that the copper precipitate at first contained more organic material, but it stood in the laboratory at 20° C. — 25° C. for 2 or 3 days and may have undergone decomposition, although this does not seem probable.

Preparation of the barium salt by direct precipitation with barium hydroxid. Twelve gm. of the crude material were dissolved in water to which a small amount of hydrochloric acid was added, and the solution boiled. Barium hydroxid sol. was now added to strong alkaline reaction. The precipitate was filtered out, dissolved in dil. hydrochloric acid sol., and reprecipitated with barium hydroxid sol. It was then purified by three alcoholic precipitations in the usual

manner. The resulting precipitate weighed, after drying, 12.8 gm. It was dried for analysis at 105° C., in vacuum, over phosphorus pentoxid. Analytic data:

0.2050 gm. gave 0.1261 gm. BaSO₄.
 0.2019 gm. gave 0.1186 gm. Mg₂P₂O₇.
 0.3348 gm. gave 0.0834 gm. CO₂ and 0.0555 gm. H₂O.
Found: Ba, 36.20%; P, 16.37%; C, 6.79%; H, 1.85%.

This method of preparation does not seem to replace entirely the bases originally present for, on fusing the salt with potassium hydroxid and potassium nitrate for the determination of phosphorus, a faint trace of green was produced, showing the presence of a trace of manganese, which was present in somewhat greater quantity in the original alcoholic precipitate.

Preparation of the barium salt by the copper acetate method. Twelve gm. of the alcoholic precipitate were dissolved in water, a few drops of hydrochloric acid sol. added, and then an excess of a conc. sol. of copper acetate. The conversion of the resulting copper salt to the barium salt was accomplished by the procedure described previously for this method. The product free from phosphates, weighed 12.2 gm. It was dried for analysis at 105° C., in vacuum, over phosphorus pentoxid. Analytic data:

0.2333 gm. gave 0.1418 gm. BaSO₄.
 0.2042 gm. gave 0.1160 gm. Mg₂P₂O₇.
 0.3342 gm. gave 0.0862 gm. CO₂ and 0.0497 gm. H₂O.
Found: Ba, 36.90%; P, 16.32%; C, 7.03%; H, 1.66%.

The material was free from manganese, and probably this method insures a more thorough separation of the bases than the former method.

Purification of the barium salt by water extraction. Ten gm. of this material were extracted with five successive 25 cc. vol. of water, being rubbed up thoroughly in a mortar with each portion. The final extract gave only a cloudiness with alcohol. The united extracts were treated with 3 vol. of alcohol and the precipitate collected, washed with alcohol and ether, and dried. Weight: 4 gm. It was dried for analysis at 105° C., in vacuum, over phosphorus pentoxid. Analytic data:

0.1974 gm. gave 0.1211 gm. BaSO_4 .

0.2068 gm. gave 0.1116 gm. $\text{Mg}_2\text{P}_2\text{O}_7$.

0.3655 gm. gave 0.0794 gm. CO_2 and 0.0629 gm. H_2O .

Found: Ba, 36.10%; P, 15.53%; C, 5.93%; H, 1.93%.

Attempt to crystallize the water-insoluble fraction. All the water-insoluble material was dissolved in 0.2 percent hydrochloric acid sol., and alcohol added until a fairly heavy amorphous precipitate was obtained. After standing several days, this precipitate had in part crystallized, but there was considerable amorphous matter mixed with the crystals. The form of the crystals was identical with that of the crystals obtained before in pure form. Upon standing for several days, complete crystallization failed to take place, and the mixture of crystals and amorphous matter was filtered out and dried for analysis at 105°C ., in vacuum, over phosphorus pentoxid. Analytic data:

0.1569 gm. gave 0.0942 gm. BaSO_4 .

0.1525 gm. gave 0.0889 gm. $\text{Mg}_2\text{P}_2\text{O}_7$.

0.2455 gm. gave 0.0570 gm. CO_2 and 0.0391 gm. H_2O .

Found: Ba, 35.35%; P, 16.87%; C, 6.33%; H, 1.78%.

None of these substances bears any resemblance to the material obtained by us as described in the second part of this paper. No satisfactory reason suggests itself for this fact, although there were three differences in the methods of preparation: first, a larger amount of alcohol was used, securing a more complete precipitation; second, tannic acid was not added to the original extract; and third, the extraction and precipitation of the compound were completed in three days, whereas two weeks were consumed for the first preparation. The latter fact could make a difference only if the material tends to decompose. Believing that these differences of procedure are not adequate to explain the disparities in composition, we leave the question open for further investigation, with the suggestion that there may possibly be differences in wheat-brans, one of the compounds being formed first and converted gradually, by the metabolism of the plant, into the other.

Conclusions. A large part of the organic phosphorus of wheat-bran exists as phytin, similar to that from many other sources. A crystalline tri-barium salt may readily be prepared from it. This material is most readily obtained by the copper acetate method.

There is, in addition, a considerable amount of another substance, very similar in composition, the barium salt of which contains only 34 percent of barium, instead of the 38 percent in barium phytate. The fact that this substance does not dialyze indicates that its molecule is larger than that of barium phytate.

There is, finally, a compound differing widely from phytin in having more carbon and less phosphorus in the molecule, which by hydrolysis splits off a reducing sugar (pentose), and whose barium salt contains only about 31 percent of barium. We do not believe the composition of this substance has been definitely fixed. It has not been obtained in crystalline form, the analogous crystalline brucine salt prepared by Anderson probably being simply brucine phosphate.

The formulas, $C_6H_{18}O_{24}P_6Ba_3$, for the tri-barium salt, and $C_6H_{24}O_{24}P_6$, for the acid, accord more closely with our analytical results than any other formulas, although the agreement is not entirely satisfactory.

Two tables of analytic results are appended.

TABLE I

	From wheat bran, found: Percent	For inosite hexa-phosphate, $C_6H_{18}O_{24}P_6$, calculated: Percent	For $C_6H_{24}O_{24}P_6$, calculated: Percent	Found by Patten and Hart: Percent	For $C_6H_{24}O_{27}P_6$, calculated: Percent
P	27.67	28.18	27.92	25.98	26.07
C	10.90	10.90	10.81	10.63	10.08
H	3.54	2.72	3.63	3.38	3.39

TABLE 2

	From wheat bran, found (crystallized sample): Percent	For tri-barium inosite hexa-phosphate, $C_6H_{12}O_{24}P_6Ba_3$, calculated: Percent	For $C_6H_{18}O_{24}P_6Ba_3$, calculated: Percent	For $C_6H_{18}O_{27}P_6Ba_3$, calculated: Percent
P	17.12	17.44	17.35	16.62
Ba	37.77	38.65	38.43	36.78
C	6.89	6.75	6.72	6.43
H	1.96	1.12	1.69	1.62

ADDENDUM

After the proof of the foregoing paper had been corrected and returned to the editor, Anderson* published a new series of papers,

* Anderson: *Jour. Biol. Chem.*, 1915, xx, pp. 463, 475, 483, 493.

in which the disagreement between his findings for wheat-bran, and those of Patten and Hart and ourselves, is explained. The existence in wheat-bran of a phytin-splitting enzyme, a "phytase," active in dilute hydrochloric acid sol., accounts fully for the failure to isolate in all cases from wheat-bran the inosite hexaphosphate compound. We refer above (p. 115), to the possible occurrence of such an agent. As to the reason why this enzyme has been active in some instances and not in others, there appear to be two possibilities. Either the hydrochloric acid used for extraction, having been made up roughly to 0.2 percent, was somewhat stronger and therefore (as has been shown) inhibitory to the phytase; or, in the case of our work, which was conducted during the winter months, the original extraction having been made in a cold room at a temperature not above 10° C., the enzyme was inactivated by the low temperature.

THE NEUTRAL-SULFUR AND COLLOIDAL-NITROGEN TESTS IN THE DIAGNOSIS OF CANCER*

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Introduction. During the past few years a number of urinary tests have been suggested for the *early* diagnosis of cancer. These tests have originated from German and Austrian laboratories; and, immediately after their publication, scientific workers in all parts of the world have endeavored to confirm or disprove the value of these tests, which, if specific, would aid greatly in the conquest of carcinoma. The reports of various observers have been either very favorable or totally discouraging. Accordingly, it is impossible to draw definite conclusions, at present, regarding the efficiency of these laboratory methods.

We have attempted to determine the relative values of the urinary colloidal-nitrogen and neutral-sulfur tests; to study the percentage of positive results obtained with these methods in known cases of malignancy; and to discover, if possible, whether the results of these tests run parallel in cancer and non-cancerous diseases.

COLLOIDAL-NITROGEN TEST. In 1892, Töpfer (1) found that the urine of patients suffering from cancer contained a very large amount of "extractive substance." This "extractive substance" was calculated by first determining the quantity of total nitrogen and then subtracting, from this amount, the sum of the nitrogen values for urea, uric acid, and ammonia, of the same urine. Bondzynski and Gottlieb (2), five years later, reported that the nitrogen in oxyproteic acid, in the urine, was 2 to 3 percent of the total urinary nitrogen. Salkowski (3), and Hess and Saxl (4), using different procedures, concluded that the oxyproteic acid portion of the alcohol-precipitable substances is increased in the urine of human beings suffering from carcinoma.

* Proceedings of the Columbia University Biochemical Association, Dec. 4, 1914; *BIOCHEM. BULL.*, 1915, iv, p. 217.

Salkowski and Kojo (5), in a preliminary communication, recently suggested several methods for the determination of colloidal nitrogen in the urine. A year later, Kojo (6) published the results of a comparative study of the various procedures suggested in this connection. Kahn and Rosenbloom (7) studied the zinc-sulfate-precipitable, colloidal, nitrogenous material from the urine of normal subjects, as well as of carcinomatous patients, and concluded that the amount of colloidal nitrogen was invariably increased in carcinoma. They also found that diseases like myocarditis, diabetes, leukemia, and anemia, likewise gave a high colloidal-nitrogen index. They concluded that this quantitative test was not specific for cancer. Kahn and Rosenbloom (8) studied the amount of colloidal nitrogen in the urine of a dog suffering from a malignant neoplasm. In this case they used dialysis as a part of the method, and found that the quantity of colloidal nitrogen was much greater in the urine of the diseased dog than the amount present in the urine of normal dogs.

Volpe (9) found that the colloidal-nitrogen index is of special value in cancer diagnosis. Mancini (10), using the Salkowski method, found that there were increased eliminations of colloidal nitrogen in the urines of patients afflicted with cancer, but this increase also occurred in pneumonia and pleurisy. Semionov (11) reported that the colloidal nitrogen output is low in normal individuals and is increased in cancer patients. He concluded that although the normal index *excludes* the possibility of a malignant growth, the increased amount of colloidal nitrogen in the urine is not specific for carcinoma. Konikov (12) found that the average amount of colloidal nitrogen in the urine, as determined by the Salkowski-Kojo method, was 1.68 percent of the total nitrogen in normal cases, and 2.47 percent in carcinomatous individuals. Of 73 cases of cancer investigated by him, only 9 showed a higher coefficient than 2.5 percent.

According to Marcel, Labbé, Dauphin (13) and others, on the other hand, increase in the urinary colloidal nitrogen is an index of a derangement of nitrogenous metabolism; and while it may serve to detect functional insufficiency in the liver, it is not at all specific for cancerous states. Carforio (14), also, concluded that the colloidal nitrogen index is not pathognomonic of cancer.

NEUTRAL-SULFUR TEST. Salomon and Saxl (15) have described a neutral-sulfur reaction in the urine. Like all the other tests in this connection, it has given excellent results in some hands but, in others, has proved valueless. The abnormal constituent in the urine of carcinomatous patients is a neutral-sulfur fraction, the sulfur of which can be split off by means of hydrogen peroxide, and can be determined as barium sulfate. Positive urines yield 0.010 to 0.018 gm. of barium sulfate from this fraction, for 100 cc. of urine. Of 41 carcinoma cases examined by Salomon and Saxl, 30 were positive, 4 faintly positive, 1 questionable, and 6 negative. Of 182 normal urines, 6 were positive, 3 faintly positive, 1 questionable and 172 negative.

Petersen (16) divided his cases into three classes. (A) Clinically non-cancerous suspects: of 26 patients examined, 25 gave a negative Salomon and Saxl neutral-sulfur reaction. (B) Clinically cancer suspects: of 20 cases examined, 5 were negative, 2 alternately positive and negative reactions, and 13 cases positive. (C) Manifest cancer: of 19 cases, 17 always gave a good positive reaction; the two negatives were icteric and cachectic. Dozzi (17) found that the test was invariably negative in all his patients free from cancer or tuberculosis, but the frequency of the positive responses in tuberculous patients detracted from its value as a sign of cancer, although cancer is rarely mistaken for tuberculosis. The only cancer cases that gave negative results were those in which the cancer had been excised. Murachi (18), also, found an increase in the neutral sulfur from cancer patients. The coefficient, according to him, may be 3.8 percent of the total sulfur.

In contrast to the foregoing, Pribram (19) found that only 60 percent of cancer patients gave a positive Salomon-Saxl test and that the test is, therefore, far from specific. Alekseev (20) came to a similar conclusion. Mazzitelli (21) has studied this test in 50 cases of cancer, with and without cachexia. Of 18 cases of the latter variety, the test was positive in 14; but also in 8 of 10 cases of tuberculous cachexia, and 16 of 23 cases of cachexia of various origins, including 11 with cancer and 4 with tuberculosis, Greenwald (22) concluded that this test has no value in the diagnosis of cancer.

Stadtmüller and Rosenbloom (23) studied sulfur metabolism, in general, in carcinoma. They found that the lowest average total-

sulfur excretion (0.88 gm. per day) occurred in a series of 13 cases of carcinoma. The same series also showed the lowest average neutral-sulfur excretion (not by the Salomon and Saxl method)—0.20 gm. per day. The proportion of neutral sulfur to total sulfur in the series was considerably higher than the normal proportion. They conclude, however, that "it is a precarious undertaking to diagnose a malignant tumor on the basis of the absolute or relative amount of neutral sulfur in the urine."

Experimental. The following methods were used by us for determinations of the colloidal nitrogen and the neutral sulfur in the urine.

COLLOIDAL-NITROGEN. The urine was first tested for coagulable protein, which, if found, was removed by means of heat coagulation, with addition to the boiling liquid of a few drops of dilute acetic acid sol. To 100 cc. of mixed, filtered, 24-hr. specimen of urine, zinc sulfate was added in sufficient quantity to effect saturation. The saturated liquid was allowed to stand for 24 hours, then was filtered through ashless paper, and the precipitate washed several times on the paper with saturated zinc sulfate solution, to remove nitrogenous substances adherent to the precipitate. The paper and precipitate were then placed in a Kjeldahl flask and the nitrogen content determined by the Kjeldahl method. The *total* nitrogen in 5 cc. of urine was also determined by the Kjeldahl method. The ratio of the nitrogen in the zinc sulfate precipitate to the total urinary nitrogen was computed.

NEUTRAL-SULFUR. The technic of the Salomon and Saxl neutral-sulfur test is the following: 150 cc. of urine, freed from coagulable protein by heat and acid, are diluted with 100 cc. of water. A mixture of 100 cc. of sat. aqueous sol. of barium hydroxid and 50 cc. of sat. aqueous sol. of barium chlorid is added, the liquid filtered and the filtrate tested with barium to see if precipitation is complete. In order to remove the ethereal sulfates, 300 cc. of the filtrate are treated with 30 cc. of conc. hydrochloric acid sol., and boiled for 15 min. in an Erlenmeyer flask, using a funnel condenser. The flask is then placed on a water-bath for 24 hr. Of the clear filtrate, 200 cc. are mixed with 3 cc. of hydrogen peroxide (perhydrol-Merck), and boiled for 15 min. with a funnel condenser. After boiling, the liquid is transferred to a conical graduate, where, at the

end of 6 hr., the amount of precipitate is observed. Antipyrin and creosote medications interfere, according to certain authors, with this test.

TABLE I
Data pertaining to normal cases

No.	Name	Diagnosis	Total N in 100 cc. urine gm.	Colloid- N in 100 cc. urine gm.	Per- cent: col- loid-N of total N	Total S in 100 cc. urine gm.	Salomon- Saxl neutral- S in 100 cc. urine gm.	Percent: neutral-S in total S
1	A. I.	Normal	0.7459	0.01006	1.35	0.112	0.0019	1.72
2	A. I.	"	0.7875	0.0098	1.25	0.109	0.0018	1.65
3	J. S.	"	0.8132	0.0109	1.84	0.097	not w'g'd	less than 1
4	M. K.	"	0.7986	0.0167	2.10	0.124	0.0027	2.07
5	D. F.	"	0.9178	0.0164	1.74	0.171	0.0033	1.94
6	J. S.	"	0.9356	0.0175	1.87	0.195	0.0026	1.34
7	S. H.	"	0.9471	0.0136	1.44	0.172	not w'g'd	less than 1
8	R. L.	"	0.7344	0.0118	1.62	0.155	not w'g'd	less than 1
9	B. C.	"	0.5467	0.0103	1.90	0.137	0.0017	1.22
10	J. H.	"	0.8264	0.0158	1.92	0.208	0.0044	2.14
11	D. F.	"	0.8326	0.0146	1.75	0.170	not w'g'd	less than 1
12	W. S.	"	0.8521	0.0113	1.33	0.115	not w'g'd	less than 1
13	M. K.	"	0.7287	0.0153	2.10	0.110	0.0025	1.75
14	M. K.	"	0.9812	0.0210	2.15	0.135	0.0023	1.90
15	J. G.	"	0.9245	0.0138	1.50	0.152	0.0024	1.62
16	A. P.	"	0.8352	0.0129	1.55	0.162	not w'g'd	less than 1
17	V. L.	"	0.6992	0.0118	1.70	0.178	not w'g'd	less than 1
18	B. H.	"	0.9272	0.0124	1.34	0.096	not w'g'd	less than 1
19	D. R.	"	0.9817	0.0124	1.35	0.087	not w'g'd	less than 1
20	S. H.	"	0.8228	0.0116	1.42	0.135	0.0025	1.90
21	R. L.	"	0.8298	0.0142	1.72	0.109	0.0020	1.85
22	M. B.	"	0.8218	0.0156	1.90	0.175	not w'g'd	less than 1

The accompanying tables present our comparative results.

Table I shows the results obtained in normal subjects. The nitrogen values for the zinc-sulfate precipitate, as compared with those for total nitrogen, varied from 1.25 percent as a minimum, to 2.15 percent as a maximum, with an average of 1.67 percent. This agrees with the results obtained by Salkowski and Kojo, and Einhorn, Kahn and Rosenbloom, who obtained respectively averages of 1.75 percent and 1.9 percent. Of 22 urines examined, 10 gave a precipitate by the Salomon and Saxl method that was so light as not to be weighable. The other 12 cases gave sulfate precipitates which varied between 1.22 percent of the total sulfur as a minimum, and 2.14 percent of total sulfur as a maximum. In general the Salomon and Saxl test was negative in all cases in which the neutral sulfur

TABLE 2
Data pertaining to cancer cases

No.	Name	Diagnosis	Total N in 100 cc. urine gm.	Colloid- N in 100 cc. urine gm.	Per cent: col- loid-N of total N	Total S in 100 cc. urine gm.	Salomon- Saxl neutral- S in 100 c.c. urine gm.	Percent: neutral-S in total S
23	T. A.	Cancer of uterus	0.9756	0.0419	4.3	0.085	0.0031	3.7
24	M. W.	Gastric cancer	1.1071	0.0636	5.75	0.087	0.0035	4.1
25	F. C.	Gastric cancer	1.1950	0.0652	4.62	0.108	0.0041	3.8
26	A. R.	Cancer of breast	1.2104	0.0568	4.7	0.152	0.0045	2.9
27	S. G.	Gastric cancer	0.9260	0.0361	3.9	0.095	not w'g'd	less than 1
28	T. S.	Cancer of liver	0.5762	0.0247	4.3	0.104	0.0035	3.4
29	T. A.	Cancer of uterus	1.3550	0.0469	4.2	0.087	0.0032	3.7
30	M. W.	Gastric cancer	0.8722	0.0305	3.5	0.1055	0.0025	2.5
31	S. G.	Gastric cancer	0.9128	0.0447	4.9	0.1243	0.0045	4.4
32	A. R.	Cancer of breast	1.0424	0.0458	4.4	0.1175	0.0037	3.2
33	A. G.	Cancer of rectum	0.4728	0.0212	4.5	0.1480	0.0050	3.4
34	C. J.	Cancer of cervix	1.1307	0.0431	4.7	0.0875	0.0030	3.5
35	W. J.	Gastric cancer	0.9246	0.0388	4.2	0.0986	0.0036	3.7
36	B. M.	Cancer of liver	1.1108	0.0377	3.4	0.097	0.0031	3.2
37	K. B.	Cancer of liver	0.8229	0.0427	5.2	0.1452	0.0062	4.3
38	E. F.	Cancer of stomach	1.1055	0.0608	5.5	0.1445	0.0059	4.1
39	B. J.	Cancer of pancreas	1.1782	0.0494	4.2	0.1378	0.0060	4.4
40	E. M.	Cancer of stomach	1.1363	0.0441	3.8	0.1025	0.0043	4.2
41	B. M.	Cancer of liver	0.8912	0.0427	4.8	0.1644	0.0070	4.0
42	C. J.	Cancer of cervix	0.7755	0.0334	4.3	0.1552	0.0067	4.5
43	P. B.	Cancer of uterus	0.5737	0.0252	4.4	0.1275	0.0049	4.1
44	M. G.	Cancer of stomach	0.9345	0.0345	3.7	0.09865	0.0035	3.4
45	E. F.	Cancer of stomach	0.8548	0.0435	5.1	0.1143	0.0038	3.5
46	M. W.	Gastric cancer	0.9845	0.0502	5.1	0.0975	0.0026	2.7
47	F. O.	Cancer of pelvis	0.9642	0.0424	4.4	0.0956	0.0039	4.2
48	M. F.	Cancer of cervix	0.8750	0.0411	4.7	0.1140	0.0031	2.9
49	C. J.	Cancer of cervix	0.6437	0.0270	4.2	0.1231	0.0037	3.1
50	R. W.	Cancer of rectum	0.8821	0.0379	4.3	0.1242	0.0041	3.4
51	C. S.	Cancer of appendix	1.0632	0.0404	3.8	0.0983	0.0031	3.2
52	R. S.	Cancer of rectum	1.0452	0.0387	3.7	0.0875	0.0031	3.5
53	C. S.	Cancer of appendix	1.2371	0.0507	4.1	0.0844	0.0031	3.7
54	Z. H.	Cancer of stomach	1.1685	0.0561	4.8	0.0847	0.0026	3.1
55	A. T.	Cancer of stomach	0.7325	0.0370	5.05	0.0934	0.0031	3.4
56	I. S.	Cancer of liver	0.4510	0.0237	5.2	0.0895	0.0029	3.3
57	A. I.	Cancer of liver	1.4784	0.0784	5.3	0.0880	0.0034	4.2
58	R. A.	Cancer of breast	0.6905	0.0359	5.2	0.0975	0.0041	4.3
59	G. T.	Cancer of esophagus	0.9075	0.0472	5.2	0.1302	0.0043	4.1
60	G. F.	Cancer of esophagus	0.6470	0.0349	5.4	0.0888	0.0031	3.6
61	M. B.	Cancer of intestine	0.6984	0.0356	5.1	0.1207	0.0055	4.6
62	B. J.	Cancer of pancreas	0.9750	0.0536	5.5	0.1405	0.0053	3.8
63	E. O.	Cancer of esophagus	1.1750	0.0564	4.8	0.0995	0.0030	3.2
64	D. S.	Cancer of breast	0.8705	0.0409	4.7	0.0894	0.0050	4.5
65	T. G.	Cancer of breast	0.6095	0.0289	4.75	0.1114	0.0051	4.7
66	G. H.	Cancer of uterus	0.5276	0.0243	4.6	0.1237	0.0039	3.3
67	I. S.	Cancer of liver	0.7654	0.0260	3.4	0.1047	0.0040	3.9
68	Z. H.	Cancer of stomach	0.8505	0.0366	4.3	0.0997	0.0038	3.8
69	A. T.	Cancer of stomach	0.9472	0.0360	3.8	0.0896	0.0029	3.3
70	B. J.	Cancer of pancreas	1.1560	0.0541	4.7	0.0945	0.0035	4.8
71	D. S.	Cancer of stomach	1.423	0.0448	3.2	0.1144	0.0047	4.2
72	T. A.	Cancer of uterus	1.2025	0.0408	3.4	0.0795	0.0024	3.1
73	M. W.	Gastric cancer	0.8701	0.0296	3.4	0.0994	0.0034	3.5
74	F. C.	Gastric cancer	0.7575	0.0271	3.6	0.0774	0.0028	3.6
75	A. G.	Cancer of rectum	1.3645	0.0525	4.6	0.0985	0.0033	3.4
76	C. J.	Cancer of cervix	0.6443	0.0248	3.4	0.0863	0.0031	3.6
77	W. J.	Gastric cancer	1.2380	0.0516	4.2	0.0784	0.0035	4.5
78	B. M.	Cancer of liver	0.9522	0.0432	5.6	0.0952	0.0027	2.8
79	E. F.	Cancer of stomach	1.202	0.0528	4.4	0.1205	0.0040	3.7
80	M. G.	Cancer of stomach	0.7540	0.0337	4.5	0.1104	0.0041	3.8
81	K. B.	Cancer of larynx	0.6884	0.0353	5.2	0.0948	0.0039	4.4

TABLE 3

Data pertaining to non-cancerous cases

No.	Name	Diagnosis	Total N in 100 cc. urine gm.	Colloid- N in 100 cc. urine gm.	Per- cent: col- loid-N of total N	Total S in 100 cc. urine gm.	Salomon- Saxl neutral- S in 100 cc. urine gm.	Percent: neutral-S in total S
82	L. S.	Lung Tbc.	0.872	0.0117	1.35	0.1409	0.0047	3.4
83	A. H.	Nephritis	0.695	0.0118	1.75	—	not w'g'd	less than 1
84	L. L.	Nephritis	0.723	0.0144	2.0	—	"	"
85	P. B.	Nephritis	0.646	0.0115	1.8	—	"	"
86	C. H.	Myocarditis	1.078	0.0363	3.4	—	"	"
87	G. J.	Myocarditis	1.055	0.0221	2.1	—	"	"
88	S. B.	Typhoid	1.072	0.0139	1.3	—	"	"
89	S. E.	Typhoid	0.9465	0.0118	1.25	—	"	"
90	B. I.	Typhoid	0.6435	0.0081	1.35	0.1875	0.0031	1.7
91	H. R.	Empyema	0.7281	0.0122	1.7	—	not w'g'd	less than 1
92	B. R.	Empyema	0.8253	0.0139	1.75	—	"	"
93	J. B.	Empyema	0.7642	0.0083	1.1	—	"	"
94	A. I.	Endarteritis obliter.	0.6895	0.0108	1.6	—	"	"
95	H. W.	Endarteritis obliter.	0.7321	0.0102	1.4	—	"	"
96	A. K.	Endarteritis obliter.	0.8218	0.0147	1.8	—	"	"
97	M. R.	Endarteritis obliter.	1.0878	0.0097	0.9	—	"	"
98	J. R.	Sarcoma of leg	1.046	0.0468	4.5	—	"	"
99	C. S.	Leukemia	1.0975	0.0239	2.2	—	"	"
100	child	Hemophilia	0.784	0.0109	1.4	0.1482	0.0033	2.4
101	C. F.	Pernicious anemia	1.095	0.0130	1.2	0.1843	0.0045	2.5
102	R. K.	Atrophic cirrhosis	0.5065	0.0106	1.8	0.1077	0.0029	2.8
103	F. G.	Atrophic cirrhosis	0.7234	0.0101	1.4	0.1255	0.0025	2.1
104	A. E.	Pneumonia	0.6546	0.0111	1.7	—	not w'g'd	less than 1
105	R. E.	Pneumonia	1.0725	0.0161	1.5	—	"	"
106	B. B.	Pneumonia	1.1347	0.0125	1.1	—	"	"
107	H. S.	Pneumonia	1.1485	0.0161	1.4	—	not w'g'd	less than 1
108	J. M.	Diabetes	1.0953	0.0466	4.25	—	"	"
109	J. A.	Diabetes	1.2075	0.0465	3.75	—	"	"
110	B. S.	Diabetes	0.9642	0.0501	5.2	—	"	"
111	I. B.	Diabetes	1.2007	0.0552	4.6	—	"	"
112	I. B.	Diabetes	0.6435	0.0290	4.5	—	"	"
113	A. K.	Syphilis	0.7114	0.0263	3.7	—	"	"
114	H. H.	Syphilis	0.7227	0.0296	4.1	—	"	"
115	S. H.	Syphilis	0.5835	0.0222	3.8	—	"	"
116	M. F.	Gastric ulcer	0.6444	0.0077	1.2	—	"	"
117	M. M.	Gastric ulcer	0.9007	0.0126	1.4	0.1586	0.0036	2.4
118	B. W.	Gastric ulcer	0.8767	0.0075	0.85	0.1755	0.0042	2.5
119	C. Z.	Gastric ulcer	0.6114	0.0109	1.7	—	not w'g'd	less than 1
120	J. J.	Gastric ulcer	0.6275	0.0100	1.6	—	"	"
121	R. K.	Gastric ulcer	0.8649	0.0130	1.5	—	"	"
122	child	Chorea	0.9653	0.0116	1.2	—	"	"
123	"	Chorea	0.7556	0.0128	1.7	—	"	"
124	P. B.	Endocarditis	1.0755	0.0258	2.4	—	"	"
125	E. L.	Tbc. of glands	0.6443	0.0141	2.2	0.1641	0.0068	4.3
126	N. R.	Lung Tbc.	0.7627	0.0137	1.8	0.1974	0.0072	3.8
127	H. F.	Lung Tbc.	1.2005	0.0281	1.4	0.1722	0.0063	3.7
128	N. K.	Lung Tbc.	0.9234	0.0139	1.5	0.1645	0.0046	2.9

was less than 2 percent of the total sulfur. Considering it from this point of view, 90.9 percent of normal cases gave a negative Salomon and Saxl reaction.

In the results for 59 urinary examinations of cases of cancer (Table 2), the colloidal-nitrogen percent was generally increased to as high as 5.75 percent of the total nitrogen, the minimum being 3.4 percent. Fifty-eight of the 59 cases of cancer gave a positive Salomon and Saxl reaction. We are doubtful whether the case in which it was negative (case 27) was one of true malignancy, the diagnosis having been made clinically.

Table 3 is the most interesting. Forty-seven cases of diseases other than cancer were studied. We obtained positive results with the colloidal-nitrogen estimations in cases of myocarditis, diabetes and syphilis. The colloidal nitrogen is constantly increased in amount in diabetics. Wallace,²⁴ basing his conclusion on the findings in only two cases, states that this increase is not constant and that there is no relationship between the colloidal-nitrogen output and the severity of the diabetes. Tuberculosis and the other diseases gave negative results. On the other hand tuberculosis, hemophilia, pernicious anemia, and atrophic cirrhosis of the liver, gave positive Salomon and Saxl neutral-sulfur reactions, whereas the other diseases reacted negatively.

General conclusion. We conclude that positive results with either the colloidal-nitrogen test or the neutral-sulfur test, alone, are not indicative of carcinoma. When performed *conjointly* on urine of the same case, however, positive results with *both* methods are strongly indicative of malignancy.

Further work along these lines is desirable.

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ACTIVE IMMUNIZATION TO HAY FEVER*

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INTRODUCTION. Hay fever, or pollinosis, is a disease which manifests itself in the spring, from the latter part of May or the early part of June to the early part or middle of July; and in the autumn, from the middle of August to the end of September or early October. It is characterized by itching of the eyes and lachrymation, sneezing, serous discharge from the nose, obstructed breathing, and itching of the palate and face. If the attack is very severe, sooner or later there is coughing, and difficult breathing accompanied by wheezing. It is caused by the action of pollen grains from flowering plants, the pollen being carried by air currents and thus inhaled. If the recipient is susceptible to a particular pollen, an attack of hay fever promptly ensues.

In 1906 Wolff-Eisner (1) suggested that this disease was a condition of anaphylaxis. Dunbar (2) has studied the subject exhaustively and claims that, besides hypersusceptibility to the pollen "toxin," there must be, in patients subject to this condition, an abnormal permeability of the skin and mucous membranes for the pollen substances. This last fact we have demonstrated to my own satisfaction by dropping a small quantity of pollen on the skin of the face, when redness and itching were soon manifest; also by dropping a minute quantity of pollen on the conjunctiva, in a very short time redness and swelling of the lids occurring.

Richét and Hericourt (3) in 1898 applied the name of anaphylaxis to a symptom complex of vomiting, diarrhea, respiratory distress, and sometimes death, which was produced in animals by a sublethal dose of toxic protein, or by a dose of non-toxic protein, fol-

* Proceedings of the Columbia University Biochemical Association, June 1, 1914; *BIOCHEM. BULL.*, 1915, iv, p. 205.

lowed in twelve days by a second dose of the same substance, which did not cause any symptoms in control animals not previously so treated. Since then much research has been conducted, and many theories suggested, regarding the mechanism of the phenomenon. Our present conception of the *modus operandi* of anaphylactic shock has been evolved from the work of Vaughan and Wheeler (4) on "split proteid," of Sleswijk (5) and others on the rôle of complement during anaphylactic shock, and that of Friedberger and Hartoch (6), and Ulrich Friedman (7), on the production of anaphylatoxin *in vitro*. These investigators have given us the following hypothesis: When foreign protein is injected into an animal, there is a production of antibody or amboceptor specific for that particular protein. This amboceptor unites with the antigen. By the action of complement in the blood, the antigen then undergoes proteolysis, the proteolytic products inducing the symptoms known as "anaphylactic shock." The antibody is formed after the first injection. If the second injection is given at the proper time, the proteolysis goes on very rapidly, with the production of fractions, or anaphylatoxin, in large proportion, and consequent pronounced symptoms.

Hay fever is due, as previously stated, to a sensitization of an individual by the conveyance of pollen contents through the respiratory tract. There must be, at the time of sensitization, an abrasion of the mucous membrane so as to make parenteral absorption possible. In all likelihood, there exists in the patient an individual susceptibility to this particular disease, which seems to have some relation to heredity, for this and other allied ailments are frequent in given families. Among our patients there are two brothers with hay fever; a brother and sister with hay fever; a woman, with hay fever, whose son suffers from asthma; two cases in which a father and one or more of his children suffer from hay fever; a young woman with hay fever who had intense eczema as a child and whose mother suffers with eczema that is rebellious to treatment.

An attack of hay fever is comparable, in effect, with the Wolff-Eisner (8) tuberculin reaction in the skin or with the Calmette (9) reaction in the eye. During the flowering season of plants, pollen is transported by air currents and is inhaled by all of us. The sus-

ceptible person becomes ill from the action of the pollen contents on his respiratory mucous membrane and the skin of the face. If a quantity of air laden with pollen is directed into the stomach or rectum, the symptoms are localized in the stomach or rectum and do not appear in the nose, eyes, mouth or face. If a large dose of pollen extract is injected subcutaneously into a susceptible individual, typical symptoms of anaphylaxis result, as has been observed in a patient to whom we administered an excessive dose of the extract. Within ten minutes thereafter, this patient felt a sense of oppression in the chest, a suffusion of the face, her breathing became labored, there was marked palpitation of the heart, and within forty-five minutes, a giant urticarial rash covered her entire body. All of the symptoms subsided within two hours and the patient felt well enough to get up. Pollen grains of many varieties are capable of producing this condition, and not all individuals are sensitive to the same pollen. Among the most common plants in this country whose pollen induces hay fever are timothy, red-top and blue grass, and ragweed and golden-rod. The grasses cause the early or spring variety, whereas ragweed and golden-rod produce the late or autumnal variety.

Our experience has been mainly with the autumnal variety of hay fever. The majority of our patients were susceptible to ragweed alone; a few were markedly sensitive to ragweed and also slightly to golden-rod.

There are three methods by which it is possible to determine which kind of pollen is operative in a given case. A drop of each of a given series of weak pollen extracts may be instilled into the lower conjunctival sac of the eye. The one which produces congestion and swelling of the caruncle and mucous membrane of the lid is the one to which the patient is sensitive. Very minute quantities of the available extracts may be injected intracutaneously and the extract of the pollen to which that patient is anaphylactic will cause swelling and redness around the point of introduction. When a very minute quantity of pure pollen is gently rubbed into a small scarification wound of the skin, a wheal will develop at and around this point of scarification, if the patient is susceptible to that pollen. Some patients are sensitive to more than one pollen; and it seems

that there may be, in some cases, a general susceptibility to all pollen, so that only when a given reaction is marked is it possible to conclude which pollen is specifically causative of hay fever in a particular case. To be sure that no other factor than the pollen causes the reaction in a given instance, it is advisable to establish a negative control by simultaneous vaccination of another patient. No swelling should occur in the control.

THEORETICAL CONSIDERATIONS. According to Rosenau and Anderson (10), Otto, (11), and others, if on the seventh, eighth or ninth day after the first injection, a massive dose of antigen is injected into the experimental animal, symptoms of anaphylaxis do not occur with a dose of antigen on the twelfth day. This refractory condition, so produced, is called *anti-anaphylaxis*. This same animal will, twenty to thirty days later, become slightly sensitive to antigen, the symptoms being mild, fatal reactions rarely occurring. The reason for this refractory condition, so produced, was revealed by the researches of Neufeld and Dold (12), Kraus (13), Ritz and Sachs (14), Izar (15), Friedberger and Mita (16), Zinsser (17) and Bordet (18), who, working on the quantities of antigen, amboceptor, and alexin, which are most favorable for the production of anaphylatoxin *in vitro*, found that large proportions of antigen as compared with the other factors inhibited the production of anaphylatoxin. They also showed that an excess of amboceptor produced the same result. In view of these facts, they concluded that the great concentration of antigen in the blood of the refractory animal inhibited the production of sufficient anaphylatoxin to cause symptoms.

Zinsser and Dwyer (19), working with typhoid anaphylatoxin, showed that guinea pigs treated with sub-lethal doses of anaphylatoxin, developed a tolerance which enabled them to resist one and one-half to two units of the poison, the tolerance developing within three days and lasting to a slight degree for as long as two months.

From the foregoing facts, it should be possible to treat patients suffering with pollinosis by one of four methods:

1. By injecting a dose of pollen extract just before the hay fever time and repeating the procedure in twenty to thirty days.

2. By injecting a large quantity of immune serum during the attack.

This we have accomplished in one of our cases. From G. G., a patient who received forty-five injections of ragweed extract, we took about two ounces of blood from a vein. After the proper precaution of a Wassermann reaction, we injected subcutaneously 8 c.c. of the serum into a patient thirteen years of age, during a violent attack of hay fever. Before the expiration of thirty-six hours all symptoms of hay fever disappeared from this little patient and no signs of the disease returned during the entire season.

3. By injecting very small amounts of pollen extract at intervals of ten days or less, so that only minute quantities of anaphylatoxin are formed and the patient's tolerance is raised.

4. By injecting very small doses of anaphylatoxin made *in vitro* to produce the same results as in method 3.

PRACTICAL CONSIDERATIONS. It has been our object to immunize our patients by injecting gradually increased doses of pollen extract to produce tolerance to the anaphylatoxin formed in the body. Beginning with 1-5 units of pollen extract, the dose was gradually increased until a local reaction appeared at the site of the injection. This dose was then continued until the patient showed no more reaction. Then the dose was gradually increased as before.

One unit of pollen toxin was the amount of antigen dissolved in 1 c.c. of extract at a dilution of 1:20,000,000.

Method of preparing vaccine. Flowers were dried, stripped from their stems, and crushed by hand. This material was then enclosed in muslin bags of suitable size and thoroughly shaken in a large bottle. This bottle contained a cheese-cloth-covered, inverted, funnel connected by rubber tubing to a suction flask with the outlet in the latter protected by a silk filter. As the fine powder was shaken from the bag into the bottle, the air current carried it into the funnel and thence into the flask, where the silk filter helped to prevent loss of pollen grains. This method was partly successful with ragweed but of no use with golden-rod.

It was thought likely that *golden-rod flowers* needed a greater pulverization to free the pollen from the anthers. Flowers were accordingly put into a ball-mill and a fine dust was obtained. Sedimentation experiments were then undertaken with this powder to determine what concentration of alcohol in water, and alcohol

with ether in water, would give the greatest concentration of pollen grains in the sediment. It was found that a 20 percent solution of alcohol in water sedimented most pollen, and that, from powdered flowers containing about 8 percent of pollen, a sediment containing 15 percent of pollen could be obtained in this way. This method is not satisfactory, however, for the reason that immersion in such solutions of alcohol, for the time required for sedimentation, ruptured the pollen grains, with consequent loss of their contents. Many pollen grains were found, by microscopic examination, to be in this condition.

The greatest concentration of pollen derived, by the suction method, was about 80 percent for the ragweed powder, in only one sample of 1.75 gm. All other samples contained 50 percent or less.

Extracts of the pollen were made as follows: It was thoroughly triturated with sand in a mortar and treated with a moderate excess of 5 percent sodium chlorid solution containing 0.5 percent of phenol to prevent putrefaction. This mixture was kept in an incubator for 72 hr. at 37° C. and then filtered. None of the extracts by this method gave the biuret reaction and few gave a positive ninhydrin reaction. The filtered extract was then precipitated with 8 parts of alcohol and filtered quickly in a Buchner funnel to avoid denaturation, if possible, of the active principle by the strong alcohol. The precipitate was promptly dried and weighed. This precipitate failed to give a biuret or ninhydrin reaction. It was partly soluble in 0.85 percent sodium chloride solution and physiologically active in very weak solutions.

A total content of nitrogen in one of the extracts of *ragweed* was 0.066 percent. This same solution, on December 20th, 1913, gave a positive ninhydrin reaction, whereas on March 24th, 1914, three months later, the test was doubtful.

The dry precipitate was dissolved in 0.85 percent sodium chlorid solution with 0.25 percent of phenol, and serial dilutions were made. With these solutions patients were treated by hypodermic injections.

The method described above for the separation of pollen grains from the flowers was cumbersome and the positive results hardly justified the time expended. But the negative outcome in this re-

spect has suggested the substitution of a method that is less laborious and time consuming, and on which we are now working. The fact that the product is not completely soluble shows that denaturation occurred. For this reason we are now endeavoring to perfect a method of extraction which will prevent such a result.

Results of treatment with the vaccine. Eleven cases were treated in 1914, before and during the season for autumnal catarrh. Six cases were treated in advance of the attack. One of these was cured for the season, four had very mild symptoms, and one was not improved. Five cases were treated during the attack. The symptoms of four subsided after one to four injections, whereas one patient received no benefit. Altogether, there were five cures for the season. In four cases there was marked improvement. In two cases there was no improvement. Of the two cases that were not improved, one had a polypoidal degeneration of the middle turbinate with underlying bone necrosis. The patient had distinct asthmatic attacks every night and it was impossible to say whether the attacks were due to his hay fever or his local nasal condition. The other was a physician who reacted to both ragweed and golden-rod pollen. He received in all thirty-three injections, alternating the ragweed extract and the golden-rod extract. He came very irregularly. It is possible that at times the treatment was too intensive. His physical condition was so poor that possibly he could not develop a tolerance.

DISCUSSION. Nine of our cases reacted to ragweed pollen and two reacted to that of both ragweed and golden-rod. Both of these latter cases received both golden-rod and ragweed antigen hypodermically. One was cured but the other was not improved. When a patient is sensitive to more than one pollen, individual doses of each extract should be administered, in order to determine when the tolerance is sufficiently raised for each. Mixing the antigen is too empirical.

There are two ways of determining when a patient has become sufficiently immune to warrant discontinuance of the treatment.

1. With the complement-fixation test.
2. From the size, intensity and duration of the wheal produced by skin scarification, at different times, namely, before and during the treatment.

Clowes (20) was one of the first investigators in this country to immunize hay fever patients with pollen extracts. He performed complement-fixation tests, before and during the treatment, and showed that an increase in antibodies is produced in a few weeks.

The scarification method is the one we have generally used to diagnose and determine the degree of immunity induced. The wheal produced by the initial vaccination is measured, its time of appearance and its duration noted. After five or six treatments the patient is revaccinated and the wheal is observed again as before, and compared with the former results. When the wheal is very small or does not appear, the patient is sufficiently immune and probably will go through the season with very mild symptoms or none at all.

Naturally the question arises whether such immunization is permanent. We believe it is safe to say that, while immunity may not be successfully carried over to the succeeding year, recurrences are much milder at least and require less re-immunization. An attack the following year can probably be overcome by a few injections.

The best time to begin treatment is probably about ten weeks before the attack may be expected to occur. Regularity of attendance at about weekly intervals is important.

We feel that cures were not accomplished in two cases because treatment was begun too early; and in two other cases, because the patients were treated too irregularly. Furthermore, it is probable that some of these cases were susceptible to pollen other than that from ragweed and golden-rod. At the time of our initial work, we were not prepared with as large a variety of pollens as we now possess for the continuance of this work.

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THE INFLUENCE OF LOW TEMPERATURES UPON ENZYMES

A review

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Introduction. The influence of low temperatures on enzymes is a subject of growing importance to the chemist, the biologist, and the bromatologist. Problems in this field may be studied from either the potential or the kinetic side; for either the resistance of an enzyme to, or its activity at, low temp. may be investigated. Various researches, conducted during the last half century, have demonstrated that enzymes survive exposure to low temp. and also act as catalysts at such temp. The reports of these researches are widely scattered in the literature; and frequently the original papers may be obtained for consultation only with difficulty. It is the purpose of this paper, which is based on primary sources, to give a résumé of our present knowledge of this subject. One section is devoted to the resistance of enzymes to low temp., and one to their activity at such temp. In the first section, the following data for each enzyme are given, so far as they have been recorded in the original literature: source of enzyme; temp., time and mode of exposure. In the second section, the data given for each enzyme, so far as recorded by the various observers, are: source of enzyme, temp. and time of incubation, substratum, degree of progress of reaction, and results of comparative experiments carried out at higher temp.

2. **Resistance of enzymes to low temperatures.** The researches reviewed below demonstrate that the following enzymes survive exposure to low temp. and again exert their usual catalytic power when brought into a suitable environment:—lipase, protease of plants, pepsin, trypsin, rennin, thrombin, zymase, invertase, maltase, diastase, inulinase, oxidase, peroxidase, catalase, and simple

and aldehyde reductase. This order will be followed in presenting the data.

LIPASE. According to Kastle and Loevenhart (1) the lipase of a pig pancreas, which had been held in cold storage at 4° C. for 7 days, retained 40 percent of its power to produce hydrolysis of ethyl butyrate. A 10 percent aqueous extract of pig pancreas was held at 1° C. for 72 hr., and a 10 percent aqueous extract of pig liver was kept on ice for 48 hr. During holding at these temp., both extracts gained in power to hydrolyze butyric ester, a zymogen having become activated.

Pennington and Hepburn (2) demonstrated the presence of active lipase in the crude abdominal fat of chickens of known history, held hard frozen for periods of 12½, 13, 16, 28, 29 and 42 months at a temp. of -9.4° to -12.2° C., and of chickens, whose history prior to freezing was unknown, kept at that temp. for periods of 54 and 89 months. The chickens held for 28 or more months were not marketable and are of scientific interest only. As the period of holding hard frozen grew longer, the activity of the lipase toward esters usually became greater, and the acidity of the crude fat increased. Apparently a zymogen became converted into its active form, thus giving rise to increased activity of the lipase. The increase in activity of the lipase, and in the acidity of the crude fat, occurred less rapidly in hard frozen chickens than in birds held at higher temp., *e. g.*, room temp. Active lipase was also found in the crude abdominal fat of a chicken kept at 0° C. for 24 hr. after death.

Pennington and Robertson (3) detected lipase in eggs which had been held at a temp. of 0° C. for 66 days.

PROTEASES OF PLANTS. Kovchoff (4) demonstrated the power of these enzymes to survive freezing. Wheat seedlings which had germinated for 17 days, excoriated peas, peas excoriated after germination for 5 days, and certain tissues of the bean, *Vicia faba*—etiolated caulis tops, etiolated leaves and green leaves—were studied separately. Each sample was frozen for 24 hr., then permitted to undergo autolysis at room temp., in the presence of toluene as a bactericide, for a period varying from 2 days to 5 weeks. The amounts of protein and non-protein nitrogen were then determined. Almost invariably the former decreased and the latter increased during the autolysis. Therefore, these proteases had survived freezing and had

converted protein nitrogen into non-protein nitrogen during the autolysis. The proteolysis was most marked in the frozen wheat seedlings kept at room temp. for 5 weeks, 48.6 percent of the protein nitrogen becoming non-protein. Microscopic examination showed the absence of bacteria during the autolysis.

PEPSIN. Pozerski (5) exposed a glycerol sol. of pepsin to the temp. of liquid air (approximately -191° C.) for 45 min. The enzyme retained almost unchanged its power to digest albumin (contained in a Mett tube) in the presence of hydrochloric acid.

TRYPsin. Pozerski (5) also exposed an aqueous sol. of trypsin (pancreatin) to the temp. of liquid air (app. -191° C.) for 45 min. The trypsin retained unaltered its power to digest albumin contained in a Mett tube.

RENNIN. Chanoz and Doyon (6) exposed samples of commercial rennet to a temp. of -180° C., obtained by means of liquid air, for periods of 1, 5, 10 and 30 min. These samples coagulated milk with the same speed as did the unfrozen rennet. The curds appeared to be entirely similar.

Pozerski (5) found that a solution of rennin, kept for more than 1 hr. at the temp. of ebullition of liquid air (app. -191° C.), retained completely its power to clot milk.

THROMBIN. From the following experiment of Chanoz and Doyon (6), the conclusion may be drawn that thrombin survives exposure to a temp. of -180° C. Fresh blood of a dog, containing 1.5 part of oxalate per 1000, was kept in liquid air at a temp. of -180° C. for 13 min. After thawing at ordinary laboratory temp., the blood coagulated upon addition of calcium chlorid, in the same manner as did an unfrozen control sample.

ZYMASE. Zymase survives exposure to extremely low temp. Buchner (7) obtained the enzyme by grinding yeast beneath a layer of liquid air. He also prepared zymase by grinding 500 gm. of Berlin bottom yeast S with its own weight of solid carbon dioxid (carbon dioxid snow) for $\frac{1}{2}$ hr.; the stone-like mass gradually becoming soft and slightly liquid. The plasma was then obtained from the triturated mass by filtration on a hardened filter with the aid of suction; it fermented sucrose. Zymase also survived complete freezing of yeast press-juice, in fact a process for the concentration of the enzyme was based on that fact. Press-juice, con-

tained in a tall glass cylinder, was completely frozen with a mixture of ice and rock salt, and the frozen mass permitted to thaw slowly but completely. During liquefaction, the juice separated into two layers; a colorless upper layer, consisting of almost pure water, and a deeply reddish-brown lower layer, the conc. press-juice. The upper layer possessed but slight fermentive power and the lower layer a fermentive power considerably greater than that of the original press-juice, the conc. juice at the very bottom of the lower layer being the most active. Sucrose was used as substrate in these tests.

Ahrens (8) concentrated yeast press-juice, in order to increase its zymase content, by cooling to a temp. not lower than -2° C., while stirring. Ice crystals, which contained but slight quantities of the constituents of the juice, separated and were removed by rapid filtration with the aid of pressure. The zymase was in the filtrate. In order to attain a still greater concentration of the enzyme, in some of the experiments, the filtrate was cooled and the entire procedure just outlined was repeated several times.

Macfadyen (9) subjected yeast-cell plasma to the temp. of liquid air, -182° to -190° C., for a period of 20 hr. After this exposure, the zymase remained unchanged in its power to produce alcohol and carbon dioxid.

INVERTASE (SUCRASE) retains its activity after exposure to the temp. of solid carbon dioxid; for the yeast-cell plasma, obtained by Buchner (7) by means of carbon dioxid snow, produced alcoholic fermentation of sucrose during incubation at 22° C.

Pozerski (5) held solutions of invertase, prepared from beer yeast and from *Aspergillus niger*, at the temp. of liquid air (app. -191° C.) for 45 min. The enzyme completely retained its power to invert sucrose.

MALTASE (GLUCASE) retains its activity after repeated exposure to a temp. as low as -2° C. The yeast press-juice, concentrated by the process of Ahrens (8) and incubated at a temp. of 5° to 18° C., with a wort prepared from starch paste and kiln-dried malt, induced alcoholic fermentation of the latter.

DIASTASE. Pozerski (5) studied the action of liquid air (temp. app. -191° C.) on solutions of diastase. Two varieties of the enzyme were used, salivary diastase contained in filtered mixed

human saliva, and amylase from *Aspergillus niger*. After the solution of each variety had been subjected to that temp. for 4-5 min., it retained unaltered its power to hydrolyze starch.

INULINASE. Pozerski (5) subjected a solution of inulinase, obtained from *Aspergillus niger*, to the temp. of liquid air (app. —191° C.) for 45 min. The inulinase completely retained its power to hydrolyze inulin.

OXIDASE. Pennington, Hepburn, St. John, Witmer, Stafford and Burrell (10) held, at 0° C., both milk and cream containing formaldehyde (0.1 percent) and which were bacteriologically sterile; the milk for 35 days, the cream for 28 days. Analyses were made at weekly intervals. During the entire period of holding, the trikresol oxidase of both the milk and the cream retained its activity.

Hepburn (11) records the occurrence of oxidases in the crude fat of a chicken held at 0° C. for 15 days after death, and in the crude fat of chickens of known history held hard frozen at —9.4° to —12.2° C. for 9 months; and also of birds, whose history prior to freezing was unknown, kept at —9.4° to —12.2° C. for periods of 23 and 63 months.

PEROXIDASE. The presence of peroxidase in the crude fat of all the chickens mentioned in the preceding paragraph was demonstrated by Hepburn (11).

CATALASE. This enzyme was detected by Hepburn (11) in the crude fat of chickens of known history kept hard frozen for 9 months at —9.4° to —12.2° C. Pennington and Robertson (3) found that, after eggs had been held at 0° C. for 65 days, the catalase of both the white and the yolk was still active.

The work of Pennington, Hepburn, St. John, Witmer, Stafford and Burrell (10) shows that the catalase in both milk and cream, rendered bacteriologically sterile by formaldehyde (0.1 percent), retained its activity during holding at 0° C. for as long as 21 days.

Van Driest (12) reports the presence of catalase in frozen sole, held at —2° to —9.5° C. for periods of 19 to 21 days, and in frozen cod kept at —2° to —6.5° C. for 30 days.

REDUCTASE. Hepburn (11) found reductases in the crude fat of chickens subjected to prolonged hard freezing at —9.4° to —12.2° C. Simple reductase, which decolorized methylene blue, was found in chickens of known history kept for 9 months, and

in birds, whose history prior to freezing was unknown, held for 23 months. Aldehyde reductase, which decolorized methylene-blue-formaldehyde sol., occurred in chickens whose history prior to freezing was unknown and which had been in a freezer for periods of 23 and 63 months.

According to Pennington, Hepburn, St. John, Witmer, Stafford and Burrell (10), the simple reductase retained its activity as long as 28 days in both milk and cream rendered bacteriologically sterile by formaldehyde (0.1 percent) and held at 0° C. The aldehyde reductase of the cream likewise remained active during that period of holding.

3. Activity of enzymes at low temperatures. Studies have been made of the activity of the following enzymes at low temp.: lipase, diastase, invertase, maltase, zymase, pepsin, trypsin, galactase, urease, rennin. This order will be followed in presenting the data. At times the enzyme studied was permitted to produce autolysis, at times to act in solution on an artificial medium, at a given low temp.

LIPASE. Kastle and Loevenhart (1) studied the influence of temp. as low as —10° C. on the lipolysis of ethyl butyrate. For lipase, 1 cc. each of 10 percent aqueous extracts of liver and pancreas from a pig were used. In each experiment, this quantity of lipase was permitted to act on 0.23 gm. of the ester, in the presence of toluene as a bactericide, the total volume being 5 cc. After a reaction period of 30 min., the percentage of the ester hydrolyzed by the enzyme was:

Temp. of the Reaction	Percentage of ethyl butyrate hydrolyzed by	
	Pancreatic lipase	Hepatic lipase
40° C.	2.82	11.29
30° C.	3.16	5.96
20° C.	2.51	5.27
10° C.	1.88	3.89
0° C.	1.25	2.26
—10° C.	—	0.70

Richardson (13) ground 150 gm. of perfectly fresh hog pancreas with 500 c.c. of water, emulsified with 3 k. of neutral lard, and stored the emulsion at a temp. of —9° to —12° C. The pancreatic lipase caused the initial acidity of 0.25 percent free acid to rise to 2.42 percent after 2 months, and to 4.30 percent after 3 months.

In the course of a study of the influence of temp. on the hydrolysis of esters, Hepburn and Pennington (14) demonstrated the activity, *in vitro*, of the lipase of the crude abdominal fat of the chicken at 0° C. and at -6.7° to -9.4° C. The increase in acidity due to the action of the lipase for 3 days, in the incubator at 40° C., was chosen as a standard for comparison. With this were compared the increases in acidity, due to the action of the lipase, for 3 days in a house refrigerator (average temp. 17.2° C.); for 18 days in a mechanically refrigerated chill room at 0° C.; and for 45 days in a mechanically refrigerated freezer at -6.7° to -9.4° C. The crude fat was extracted with ten-fold its weight of water, and 50 cc. of the extract were permitted to act on 1 cc. of an ester. The ratios of increase in the acidity of the substrates were expressed throughout on a basis of the action of the enzyme for a period of 3 days, and the following data were obtained.

The lipolysis of ethyl acetate in the incubator was twice as rapid as in the refrigerator, 15 times as rapid as in the chill room, and $37\frac{1}{2}$ times as rapid as in the freezer. Ethyl butyrate was hydrolyzed by lipase in the incubator $2\frac{1}{2}$ times as fast as in the refrigerator, 12 times as fast as in the chill room, and 40 times as fast as in the freezer. Ethyl benzoate was split by the enzyme in the incubator $8\frac{1}{2}$ times as rapidly as in the refrigerator, $25\frac{1}{2}$ times as rapidly as in the chill room, and 255 times as rapidly as in the freezer. The hydrolysis of amyl salicylate by lipase in the incubator was $6\frac{1}{2}$ times as rapid as in the refrigerator, 13 times as rapid as in the chill room, and $97\frac{1}{2}$ times as rapid as in the freezer.

Although the rate of lipolysis was decreased by a lowering of the temp., lipolysis took place even at the temp. of the freezer, while the reaction mixture was frozen solid.

DIASTASE. Müller (15) prepared a glycerol solution of diastase from the liver of the carp, and used 1 percent starch paste as substrate. The volumes of enzyme extract and starch paste were kept constant in the entire series of experiments. The opalescence of the mixture vanished after digestion for $1\frac{1}{4}$ min. at 25° C., for 5 min. at 8° C., or for 20 min. at 0° C. The solution then reacted violet to iodine. The solution first gave a red color with iodine after $3\frac{1}{4}$ hr. at 25° C., 18 hr. at 8° C., or 32 hr. at 0° C. The solution lost its power to react with iodine after digestion for $8\frac{1}{2}$ hr. at

25° C., 44 hr. at 8° C., or 72 hr. at 0° C. Therefore, even at the latter temp., diastase transformed starch through the stages of soluble starch and erythrodextrin to maltose.

INVERTASE, MALTASE, ZYMASE. These enzymes are active at the temp. of an ice box, for, according to Buchner (16), yeast press-juice produced alcoholic fermentation of sucrose, maltose, glucose, and fructose at that temp.

PEPSIN (GASTRIC PROTEASE). Murisier and Fick (17) extracted finely divided gastric mucosae from various animals with 40-fold their weights of water, and permitted these extracts to act at various temp. on small cubes of coagulated albumen in the presence of hydrochloric acid. The extracts of the gastric mucosae from pigs and dogs rarely acted upon coagulated albumen at temp. below 10° C. and never acted, even in the slightest degree, at 0° C. On the other hand, pepsin extracts prepared with gastric mucous membranes from frogs, pike and trout, regularly digested coagulated albumen at 0° C., and were fully as active at 40° C. as were the extracts obtained with mucosae from pigs and dogs. From these experiments, which were qualitative, Murisier and Fick concluded that the gastric protease of cold blooded animals is not completely identical with that of warm blooded animals.

This opinion was shared by Hoppe-Seyler (18), who studied the action, upon fibrin shreds, of artificial gastric juice prepared with mucous membrane from pike stomachs. The optimum temp. for digestion was approximately 20° C. Proteolysis was more rapid at 15° C. than at 40° C., and became somewhat less rapid when the temp. was reduced from 15° C. to several degrees above 0° C. Digestion was very energetic at temp. between 5° and 20° C.

Flaum (19), however, demonstrated that at 0° C. the pepsin of warm blooded animals gives rise to complete proteolysis of ovalbumin, and that the same products are formed as at higher temp., although digestion is greatly retarded. He studied the action of artificial gastric juice, prepared from gastric mucosae of swine, on coagulated egg white at various temp. between 40° C. and 0° C. In all the experiments of a given series, the volume of gastric juice and the quantity of substrate were kept constant. In the preliminary series, the artificial gastric juice had been rendered free from acid metaprotein. The time required for the appearance of acid

metaprotein in the reacting mixture was: at 40° C., 1½ to 2 hr.; at 16.5° C., 2¼ hr.; at 10° C., 3 to 3¾ hr.; at 5 to 6° C., 8 hr.; at 0° C., 2 to 3 days.

In Flaum's final series of experiments, the artificial gastric juice was prepared with mucous membranes from the fundus of pig stomachs, and purified until free from proteoses and peptones. In this series, the study of proteolysis was continued until the acid metaprotein disappeared. At 40° C., decomposition of the coagulated protein began in 30 min. After 50 min., soluble protein was present; after 2 hr., acid metaprotein. Traces of proteoses were noted after 2 hr. and peptone was present after 2¼ hr. Acid metaprotein disappeared after 48 to 50 hr. At 16° to 17° C., acid metaprotein formed after 2¼ hr., and proteoses and peptone after 2½ hr., while 4 days (about 94 hr.) were required to carry digestion to the stage at which acid metaprotein was no longer present. At 10° to 10.5° C., after the lapse of 4 to 5 hr., acid metaprotein made its appearance. After 5½ to 6 hr., proteoses and traces of peptone were detected; and acid metaprotein disappeared at the end of the 5th day. At 5° to 6° C., 8 to 10 hr. were required for the formation of acid metaprotein, and about 20 hr. for the definite appearance of proteose and peptone, while 7 to 8 days elapsed before acid metaprotein completely vanished. At 0° C. in 3 to 4 days the coagulated protein was decomposed with the formation of acid metaprotein, proteose and peptone; after 14 to 15 days, acid metaprotein was no longer present.

Flaum also prepared an artificial gastric juice from frog stomachs by extraction with 2 percent hydrochloric acid sol. at 0° C. This juice digested both fibrin and ovalbumin at 0° C., and at 16° to 17.5° C. (room temp.). However, when the stomachs of living frogs were flushed, and coagulated egg white was then introduced, no digestion of the protein took place in the living animals held at 0° C. for as long as 14 days, or at 4° to 5° C. for 6 days. Digestion occurred in 1 day in frogs held at 10° C. The failure of digestion to occur *in vivo* at the lower temp. is ascribed by Flaum to the fact that no gastric juice was secreted, the lower temp. limit for its secretion being 8° C.

Müller (15) permitted the gastric protease of pike to act on 100 mg. of heavy fibrin particles. The same volume of enzyme extract

was used throughout each series of experiments, and the time of digestion was noted. A glycerol extract of the gastric mucosæ required 40 min. at 24° C., 100 min. at 8° C., and 230 min. at 0° C. An extract of the gastric mucous membrane in 0.25 percent hydrochloric acid sol. was more active, and required 20 min. at 24° C., 65 min. at 8° C., and 140 min. at 0° C., for digestion of the fibrin.

Oguro (20) studied the influence of temp. as low as 0° C. on the peptic digestion of ricin. From 0.05 to 1.0 cc. of 0.1 percent sol. of pepsin was permitted to act on 2 cc. of 0.2 percent suspension of ricin in the presence of 0.5 cc. *n*/10 hydrochloric acid sol.; the total volume of the reacting mixture always being made 4.5 cc. by dilution with water. The temp. of incubation were 38°, 20° to 21°, 8°, 5° and 0° C. The progress of digestion was followed by noting the degree of cloudiness of the suspension from time to time, the results being recorded as "clouded," "a little clouded," "traces of cloud," "almost clear," "nearly clear," "clear." The pepsin digested the ricin at all the temp. of incubation, gradually producing a clear solution, although the digestion proceeded more slowly at the lower temp. Thus, when 0.1 cc. of the pepsin sol. was used, the ricin suspension became clear after 50 min. at 38° C., 50 min. at 20° to 21° C., or 24 hr. at 0° C., while merely a trace of a cloud remained after incubation for 2 hr. at 8° C., or for 2 hr. at 5° C.

TRYPSIN. Müller (15) prepared a glycerol extract of trypsin from the intestinal tracts of carp, a fish which is without a stomach and secretes no peptic enzyme. Pieces of nutrient gelatin of equal size, and fibrin particles, were used as substrates. A given volume of the trypsin sol. dissolved the piece of gelatin after incubation for 2¾ hr. at 20° C., 16 hr. at 8° C., or 34 hr. at 0° C. The fibrin particles (100 mg.) were dissolved by a given volume of enzyme extract after digestion for 5¼ hr. at 20° C., 31 hr. at 8° C., or 72 hr. at 0° C. The trypsin, therefore, showed a distinct activity at 0° C.

GALACTASE. Babcock (21) and his collaborators (22) have demonstrated the activity of galactase, the native trypsin-like, proteolytic enzyme of milk, during the ripening of Cheddar cheese at low temp. Fresh Cheddar cheese were carried at a temp. of 25 to 30° F. for periods of 14 and 17 months. Progressive increase occurred in the total soluble nitrogen of the cheese during holding. In another series of experiments, Cheddar cheese were manufactured

with 3, 6 and 9 ounces of rennet per 1000 pounds of milk, and were permitted to ripen at temp. of 15°, 33°, 40°, 50° and 60° F. for periods of 6, 10, 12, and 14½ months. Total soluble nitrogen increased progressively in the cheese held at each of these temp., the increase being more marked at any given time in a cheese kept at a higher temp. than in one held at a lower temp. A marked increase in soluble protein was noted even in cheese stored at a temp. below freezing (15° F.). At a given temp. and in given time, a greater rise in the total soluble nitrogen occurred when larger quantities of rennet had been used in the process of manufacture. This influence of the rennet was due to the pepsin content of the latter, and was observed in the samples held at 15° and 33° F. as well as in those stored at higher temp.

The action of the galactase at all the temps., including 15° and 33° F. was absolutely demonstrated by progressive increase in amino-acid nitrogen. This increase was independent of the quantity of rennet used in the process of manufacture, and became more marked as the cheese were carried at a higher temp.

Ravenel, Hastings and Hammer (23) held a sample of "barn milk," the best milk obtainable, and one of a fair grade of dairy milk at —9° C. for 203 days. At the end of that period the water soluble nitrogen, expressed as percent of the total nitrogen of each sample, was: barn milk, 17.97 percent; dairy milk, 22.38 percent; while the average for fresh milk is 10 percent. The higher percentage in the milks subjected to prolonged holding at this low temp. is said to be due, probably, to the action of galactase.

Pennington, Hepburn, St. John, Witmer, Stafford and Burrell (10) held a milk, rendered bacteriologically sterile by formaldehyde (0.1 percent), at 0° C. for 35 days, and studied the partition of the nitrogen at intervals of 7 days. The nitrogen present as lactalbumin and syntonin, and as peptone, tended to decrease but the caseose nitrogen and the amino-acid nitrogen increased, the casein nitrogen remaining practically constant. Proteolysis was due mainly, if not entirely, to the action of galactase.

UREASE. Van Slyke (27) demonstrated that the urease of the soy bean hydrolyzes urea at temperatures as low as 0° C. The temperature coefficient of the reaction was found to be 2.80 for

the interval 0° C. to 10° C., while it remained nearly constant, with an average value of 1.91, for each interval of 10° between 10° C. and 50° C.

RENNIN. Selmi (24) noted that small quantities of rennin coagulated milk, stored at 1° to 2° C., within 4 to 5 days.

Camus and Gley (25) demonstrated that rennin exerts some action on the casein of milk at 0° C. When rennin and milk were mixed and held at that temp. for periods of $\frac{1}{2}$ hr. or longer, no precipitation of protein took place. Either lactic, acetic or hydrochloric acid was then added in quantity insufficient to produce a precipitate of protein, yet such a precipitate immediately formed, showing that the rennin had given rise to the conversion of casein into paracasein, the first stage of the enzymic curdling.

Morgenroth (26) states that, even after the prolonged action of very great quantities of rennet on milk at 0° C., the milk is not curdled; however, it clots immediately if this mixture be brought to a higher temp. The rennin, therefore, produces certain chemical changes in milk during holding at 0° C., but the definite transformation of the casein into its insoluble modification occurs only at higher temp.

Müller (15) prepared a sol. of rennin by extraction of the gastric mucosae of pike with 0.25 percent hydrochloric acid sol. Five drops of this extract were able to curdle 5 c.c. of unboiled milk in 2 min., at 40° C.; in $5\frac{1}{2}$ min., at 25° C.; in 25 min., at 15° C., and in 18 hr., at 7° C. At 0° C. no distinct curdling occurred, but a finely flocculent condition of the milk was noted after incubation for 5 days at that temp. This flocculation was apparent when the tube was slowly moved to and fro.

The time of curdling of milk is the sum of two factors, the time required for the conversion of casein into paracasein and that required for the deposition of a visible coagulum; and the latter phenomenon may require several days at lower temp., while occurring in a few min., at most, at higher temp. Experiments were carried out in such a manner that the first stage, or formation of paracasein, occurred at 0° C., while the second stage, or separation of a visible coagulum, followed at a higher temp. (40° C.). Both the diluted rennin sol. and the milk were cooled at 0° C. Several

tubes were then prepared, each containing 1 c.c. of the enzyme sol. and 5 c.c. of the substrate. The tubes were incubated at 0° C. for periods differing between 0 min. and 96 hr., then were held at 40° C., and the time required at the latter temp. for the production of a coagulum was noted, with the following results:

Time of incubation, at 0° C. Minutes	Time required for subsequent coagulation, at 40° C. Minutes
0	7
10	6, 30 sec.
20	5, 30 sec.
30	4, 45 sec.
45	4
60	2
75	1, 50 sec.
90	1, 45 sec.
105	1, 10 sec.
120	55 sec.
150	45 sec.
(Hours)	
24	45 sec.
48	45 sec.
96	45 to 50 sec.

In the last experiment of this series—held at 0° C. for 96 hr.—the flocculent precipitate was so finely divided that the period of time required for its separation at 40° C. was determined with difficulty.

Another series of experiments was carried out as described above, with the single exception that the temp. of mixing the rennin and the milk, and of the preliminary holding, was 15° C. In these experiments the transformation of the casein into paracasein proceeded more rapidly than at 0° C., and consequently less time was required for the separation of a visible precipitate. For instance, after holding at 15° C. for periods of 0, 10 and 30 min., the coagula were formed at 40° C. after 6 min., 2½ min., and 55 sec., respectively; while after 45 min. at 15° C. coagulation had already occurred.

Müller concludes that rennin exerts its characteristic action, to a certain degree, at 0° C.

4. **Summary.** The power to survive prolonged exposure to

low temp. is possessed by various enzymes, including those producing hydrolysis of fats, carbohydrates, and proteins; those concerned in biochemical oxidations and reductions; the clotting enzymes; and that of alcoholic fermentation. The enzymes retain their catalytic power after exposure, either *in situ* or in solution *in vitro*, to temp. varying from a few degrees above 0° C. to the temp. of liquid air (-180 to -191° C.). The shortest periods of holding—invariably less than 1 day and usually less than 1 hr.—were at the temp. of liquid air. The longest period of holding was 89 mo. at a temp. of -9.4° to -12.2° C.

The activity of certain of these enzymes, including rennin, zymase, and those hydrolyzing fats, carbohydrates and proteins, has been studied at low temp., varying from that of an ice box to one of -9° to -12° C. While the enzymes induce autolytic digestion, or act on artificial media, at these temp., the velocities of their reactions are always diminished to a considerable degree.

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PLANT PIGMENTS

The chemistry of plant pigments other than chlorophyll¹

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Accompanying chlorophyll in the chloroplasts of green plants and leaves there are two yellow pigments, carotin and xanthophyll. Isomers of each of these have been found in lycopin, the coloring matter of the tomato; and lutein, the pigment found in the yolk of eggs. Fucoxanthin, a xanthophyll-like substance, found in brown algae, has also been described.

The principal result of the studies thus far made upon these pigments is that a satisfactory method for their isolation and purification has been worked out. Very little if anything is known concerning their constitution. Owing to the difficulty of obtaining them in large quantities, to their ease of oxidation during the process of purification, and to the fact that upon decomposition they yield only amorphous products, it may be a long time before their constitution is established.

Carotin. Carotin is widely distributed, being generally associated with chlorophyll in the chloroplasts. It is also found in various parts of many plants. The color of yellow or orange petals is frequently due to it, *e. g.*, the corona of the common narcissus. It is largely responsible for the color of the carrot root, being present as innumerable small intracellular crystals. The tint of many fruits is due to amorphous granules of carotin.

The most recent chemical study of carotin has been made by Willstätter,² who isolated it from the leaves of stinging nettle and from carrots, and showed the complete identity of the two prepara-

¹ A review of recent work on the chemistry of chlorophyll will be found in the *BIOCHEMICAL BULLETIN*, iii, pp. 229-258, 1914. These two reviews include all the work on plant pigments published by Willstätter and his pupils. *A later review will discuss the work on flower pigments, the anthocyanins and related compounds.*

² Willstätter and Mieg: *Ann. d. Chem.*, 355, 1, 1907. Willstätter and Escher: *Ztschr. f. physiol. Chem.*, 64, 47, 1910; 76, 214, 1912. Escher: *Ibid.*, 83, 198, 1913.

tions. The following methods were used: 100 k. of dry leaves were extracted with 120 l. of petroleum ether (b. p. 40°–70°) in the cold for two days, the extract filtered off and the residue washed with 60 l. of petroleum ether. The extract was shaken with a little conc. alcoholic potash sol. to remove chlorophyll, then with water, concentrated to about 3 l. and allowed to crystallize. After shaking with 1 l. of low-boiling petroleum ether, the product was purified by repeated precipitation from carbon disulfid sol. with absolute alcohol. Finally it was recrystallized from petroleum ether (b. p. 30°–50°). The yield was 3.1 gm.–0.03 gm. per k. of dry leaves.

Data pertaining to some plant pigments

	Carotin, C ₄₀ H ₅₆	Lycopin, C ₄₀ H ₅₆	Xanthophyll, C ₄₀ H ₅₆ O ₂	Lutein, C ₄₀ H ₅₆ O ₂	Fucoxanthin, C ₄₀ H ₅₄ O ₆
Appearance.	Copper colored, rhombic leaflets.	Carmine-red, long pointed prisms or needles.	Pleochroic, dark reddish-brown plates or prisms.	Brownish-yellow plates or leaflets.	Needles.
Color by transmitted light.	Red.	Brownish to carmine-red.	Yellow to orange.		
Melting point	167.5–168°	168–9°	172°	195–6°	159.5–160.5°
Solubility in petroleum ether.	Appreciably soluble (1 g. in 1.5 l., boiling).	Slightly soluble (1 g. in 10–12 l., hot solvent).	Insoluble.	Insoluble in cold.	
Alcohol.	Practically insoluble in cold; very sparingly in hot.	Slightly soluble.	Sparingly soluble in cold; fairly readily in hot. 1 g. in 700 c.c. of hot methyl alcohol.	1 g. in 1 l. of boiling methyl alcohol.	100 gm. of methyl alcohol dissolves 0.41 gm. at 0°; 1.66 gm. at boiling temperature.
Acetone.	Very sparingly soluble.		Readily soluble.		
Carbon disulfid.	Very readily soluble.	1 g. in 50 c.c., at room temperature.	Sparingly soluble.	1 g. in 400 c.c., warm	Fairly soluble.
Ether.	1 g. in 900 c.c., hot.	1 g. in 3 l., hot.	1 g. in 300 c.c.	Easily soluble.	Slightly soluble.

Modifications of the above method, in which mother liquors from the preparation of chlorophyll were used, are given by Willstätter and Stoll.³ The carotin was found in the petroleum ether

³ Willstätter and Stoll: Untersuchungen über Chlorophyll, p. 239.

mother liquor, from which it was precipitated by alcohol. This gave a much larger yield, 0.15–0.20 gm. per k. of dry leaves.

The preparation from carrots was carried out by extracting the dry material with petroleum ether in a percolator and purifying as above; 5000 k. of fresh carrots (473 k. of dry material) gave 125 gm. of pure carotin.

Carotin forms quadratic or four-sided reddish-yellow plates, which exhibit the phenomenon of dichroism, being orange-red by transmitted light and greenish-blue in refracted light. Dilute solutions are yellow, conc. solutions orange-red but solutions in carbon disulfid, or in other solvents upon the addition of carbon disulfid, are red. Analyses of carefully purified products indicate the formula $(C_5H_7)_x$, which, from molecular weight determinations, becomes $C_{40}H_{56}$. Earlier workers gave C_5H_8 ,⁴ $C_{18}H_{24}O$,⁵ $C_{26}H_{38}$ ⁶ and other formulae.⁷ It should be mentioned that in the precipitation with absolute alcohol, a product is obtained which contains from $\frac{1}{2}$ to $\frac{2}{3}$ of a molecule of alcohol; this may be removed by recrystallization from petroleum ether. With conc. sulfuric acid it gives an indigo-blue color; upon diluting this solution, green flakes precipitate. Solutions of carotin readily absorb oxygen. Willstätter found that carotin took up 34.3 percent of its weight (11 atoms), forming a colorless compound. Various other values, from 21 percent to 37.8 percent, have been given. Shaken with $\frac{1}{3}$ of its weight of iodine in ether, a di-iodid is formed, $C_{40}H_{56}I_2$; rosettes of dark violet prisms. However, if benzene, carbon disulfid or carbon disulfid-ether is used, and a larger amount of iodine, a tri-iodid⁸ results; dark violet leaflets, melting at $136-7^\circ$. Carotin, shaken with bromine at 0° , and then allowed to stand at room temperature, forms a bromid, $C_{40}H_{36}Br_{22}$, decomposing about $171-174^\circ$. During the process, about 20 molecules of hydrobromic acid are evolved, so that probably 2 atoms of bromine are added and 20 atoms of hydrogen substituted by bromine.

⁴ Zeise: *Ann. d. Chem.*, 62, 380, 1847.

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⁷ Immendorf: *Landwirtschaftliche Jahrb.*, 18, 507, 1889.

⁸ Arnaud: *Compt. rend. acad. sc.*, 102, 1119, 1886. Willstätter and Escher: *Ztschr. f. physiol. Chem.*, 64, 59, 1910.

Carotin is of interest because of its probable physiological significance. The work of Tammes⁹ and Kohl¹⁰ shows that carotin absorbs certain rays of radiant energy, which can be made use of in photosynthesis. It may also be of importance in respiration, acting in a manner comparable to the hemoglobin of the blood. Palladin¹¹ supposes that, by the action of an oxidase, carotin is changed into xanthophyll ($C_{40}H_{56}O_2$), which in turn is acted upon by a reductase, yielding carotin. In cases where large amounts of carotin occur in organs of storage, such as the roots of the carrot, it may be of value as a reserve food material. Finally, where the colors of flowers are due to its presence, it is of importance in floral biology.

Experiments by Iwanowski,^{11a} in which chlorophyll solutions containing various amounts of yellow pigments (carotin and xanthophyll) were subjected to the action of sunlight, show that with the increase of the relative content of yellow pigments the stability of the chlorophyll towards light also increased. While this protective action is exercised by both carotin and xanthophyll individually, a more favorable effect is obtained by a mixture of the two. This action is probably due to the absorption of the blue and, especially, the violet rays, whose chlorophyll-destroying power is very high. It is not yet established whether the oxygen absorption of these pigments plays a rôle in this process.

Mention may be made here of the recent studies of Palmer and Eckles¹² on carotin and xanthophyll. They have shown that the fat of cow milk owes its natural yellow color to the presence of carotin and xanthophyll (principally carotin), which are taken up from the food and subsequently secreted in the milk fat. The same pigments are found in the body fat, blood serum, corpus luteum, and human milk. Carotin is assimilated from the food of the cow in preference to xanthophyll, partly because of its greater stability toward the digestive juices.¹³ It probably forms by far the greater part

⁹ Tammes: *Flora*, 87, 205, 1900.

¹⁰ Kohl: *Ber. d. deutsch. bot. Gesellsch.*, 24, 222, 1906.

¹¹ Palladin: *Ibid.*, 26a, 125, 378, 389, 1908; 27, 110, 1909.

^{11a} Iwanowski: *Ibid.*, 31, 600, 613, 1913-1914.

¹² Palmer and Eckles: *Jour. Biol. Chem.*, 17, 191, 211, 223, 237, 245, 1914; *Research Bulletin*, No. 10, Missouri Exper. Station.

¹³ Cf. Willstätter and Miege (2), who state that xanthophyll is very sensitive to acids.

of the lipochrome of the cow body, chiefly on account of its ability to form a compound with one of the proteins of the blood. Xanthophyll apparently is not capable of forming such a complex. It is much more soluble in bile than carotin¹⁴ which accounts for its appearance in the fat of the blood. While Palmer did not isolate carotin, it has been separated by Escher,¹⁵ who obtained 0.45 gm. of pure pigment from 10,000 cow ovaries (corpus luteum). Carotin has also been isolated from brown algae.¹⁶

Lycopin. Lycopin is the coloring matter of the tomato. Earlier investigators considered this pigment identical with carotin.¹⁷ Schunck,¹⁸ by a careful spectro-analysis of the two compounds, showed that they were quite different and gave the tomato pigment the name lycopin. The next year Montanari¹⁹ confirmed the observations of Schunck; he recognized it as a hydrocarbon and ascribed to it the formula, $C_{52}H_{74}$.

Willstätter and Escher²⁰ found that it was isomeric with carotin, having the formula, $C_{40}H_{56}$. They used tomato conserve instead of the fresh fruit for the preparation of the pigment. The conserve was treated with 96 percent alcohol (to coagulate it), pressed, dried and extracted with carbon disulfid. The concentrated extract was precipitated with absolute alcohol and then recrystallized several times from petroleum ether and carbon disulfid. The yield was about 0.2 percent of the dry substance, *i. e.*, 74 k. of conserve (5.6 k. of dry powder), yielded 11 gm. of pigment.

Lycopin forms light, or dark carmine-red, long, microscopic prisms or hair-like needles, which cannot be mistaken for carotin. Dilute sol. in carbon disulfid have a *bluish-red* color, while those of carotin have a *yellowish* tinge. The two pigments show the same color reactions with sulfuric and nitric acids. Lycopin differs from carotin in the following points: Lycopin absorbs oxygen more rapidly and to a greater extent than does carotin. Under

¹⁴ Cf. Fischer and Rose: *Ztschr. f. physiol. Chem.*, **88**, 331, 1913.

¹⁵ Escher: *Ibid.*, **83**, 198, 1913.

¹⁶ Willstätter and Page: *Ann. d. Chem.*, **404**, 237, 1914.

¹⁷ A. Arnaud: *Compt. rend. acad. sc.*, **102**, 1119, 1886. Kohl: Carotin und seine physiologische Bedeutung in der Pflanze, p. 41.

¹⁸ Schunck: *Proc. Royal Soc.*, **72**, 165, 1903.

¹⁹ Montanari: *Le stazioni sperm. agr. ital.*, **37**, 909, 1904.

²⁰ Willstätter and Escher: *Ztschr. f. physiol. Chem.*, **64**, 47, 1910.

the same experimental conditions (in 10 days), lycopin absorbed 30 percent of the oxygen from the air; carotin 0.25 percent. Lycopin does not give a crystalline iodine addition product, but a dark green amorphous product with indefinite iodine content. It reacts with bromine with the evolution of hydrobromic acid, but differs from carotin in that it takes up far more bromine than corresponds to the hydrobromic acid evolved; the compound formed is probably $C_{40}H_{44}Br_{26}$. It is very evident from these differences that the two isomers must vary considerably in structure.

Xanthophyll. The existence of a second class of yellow pigments in leaves was first mentioned by Stokes,²¹ who supposed the existence of two xanthophylls. Sorby²² believed that there were three such compounds. Borodin²³ divided the yellow pigments into two classes: the carotins, soluble in benzene and slightly soluble in alcohol; and the xanthophylls, slightly soluble in benzene but soluble in alcohol. His observations were confirmed by Monteviede,²⁴ Tschirch,²⁵ Tswett,²⁶ and Schunck.²⁷ Other writers thought that carotin was the only yellow pigment accompanying chlorophyll.²⁸ The question was partially settled by the isolation and analysis of a crystalline representative of the second class of Borodin, by Willstätter and Mieg.²⁹ The high yield of the two pigments, carotin and xanthophyll, makes it very improbable that there are any other carotinoids (this term includes both classes of pigments) accompanying chlorophyll in the land plants. Tswett,³⁰ on the basis of a chromatographic adsorption analysis (the pigments in organic solvents, filtered through a column of calcium carbonate, inulin or sugar, are adsorbed in different zones; each zone is considered a chemical

²¹ Stokes: *Proc. Royal Soc.*, 13, 144, 1864.

²² Sorby: *Quart. Jour. Science*, 8, 64, 1871; *Proc. Royal Soc.*, 21, 442, 1873.

²³ Borodin: *Melanges biologiques tires Bull. de l'Acad. Imper. de St. Petersburg*, 11, 512, 1883.

²⁴ Monteviede: *Acta Horti Petropolitani*, 13, 148, 1893.

²⁵ Tschirch: *Ber. d. deutsch. bot. Gesellsch.*, 14, 176, 1896; 22, 414, 1904.

²⁶ Tswett: *Ibid.*, 24, 316, 384, 1906; 29, 630, 1911.

²⁷ Schunck: *Proc. Royal Soc.*, 63, 389, 1898; 65, 177, 1899; 72, 165, 1904.

²⁸ Immendorf, *loc. cit.*, p. 18. Molisch: *Ber. d. deutsch. bot. Gesellsch.*, 14, 18, 1896. Tammes, *Flora*, 89, 205, 1900.

²⁹ Willstätter and Mieg: *Ann. d. Chem.*, 355, 1, 1907.

³⁰ Tswett: *Die Chromophylle in der Pflanzen- und Tierwelt*, 1910, p. 233 (Warsaw).

substance, the test being a different adsorption spectrum) distinguishes four xanthophylls, α , α' , α'' , and β . He believes the xanthophyll of Willstätter and Mieg is an isomorphous mixture of two or three xanthophylls, with the α -form predominating. Unfortunately the method does not seem to permit of the isolation of the individual pigments in quantity large enough for chemical investigation. It is entirely possible that Tswett is right and that there is present in the chloroplast a mixture of very similar isomorphous and isomeric xanthophylls, for the separation of which we have as yet no preparative method. This is all the more plausible when we consider the slight differences between carotin and lycopin; and the similarity of xanthophyll and lutein (described below). On the other hand, these compounds are rather easily oxidized and the slight differences in the absorption spectra may be due to changes in the xanthophyll by oxidation.

Xanthophyll is found in the alcoholic extract of the leaves. Attempts to obtain it pure, in which the chlorophyll was isolated as the magnesium-free derivative, pheophytin, by treatment of the extract with oxalic acid, always gave negative results. This is probably because the xanthophyll is changed by the acid into a more easily soluble and non-crystalline substance. Better results were obtained when the mother liquor of potassium chlorophyllin was used. For example, the extract from 100 k. of nettle leaves, after removal of the potassium salt by filtration and further precipitation with a large quantity of ether, was washed free from alcohol with water, the deep yellow ether sol. evaporated to about 6 l., washed repeatedly with alcoholic potash sol. and water, dried with sodium sulfate and mixed with 2 vol. of petroleum ether. The xanthophyll was purified by extraction with 1200 cc. of boiling acetone, and precipitated with 2 vol. of methyl alcohol. Recrystallized from methyl alcohol, about 12 gm. of xanthophyll were obtained.

Willstätter and Stoll have also described methods for the preparation of xanthophyll from the mother liquors of chlorophyll and of crystalline chlorophyll. These depend upon the removal of xanthophyll, from the petroleum ether sol., with dilute methyl alcohol (80-90 percent), the carotin and chlorophyll remaining in the petroleum ether. This is also the basis of the quantitative estimation of the various plant pigments. The two yellow pigments make up from 0.1 to 0.2 percent of the dry weight of the leaf, of which

xanthophyll is 0.07 to 0.12 percent and carotin 0.03 to 0.08 percent, or about 1 molecule of carotin to 1.5 to 2 molecules of xanthophyll.

Xanthophyll has the formula, $C_{40}H_{56}O_2$, and thus may be considered an oxid of carotin. Nothing is known of the function of the oxygen atoms; they are considered ether-like, since xanthophyll does not give a reaction for $\equiv COH$, $=CO$ or $-COOH$. It appears to give a very easily dissociable addition product when an ether solution is treated with methyl alcoholic potash. It shows a tendency to crystallize with alcohol of crystallization and is best obtained solvent-free by precipitation from chloroform with petroleum ether. The typical crystal forms are long tables and prisms, which are pleochroic and often show a steel blue luster. In transmitted light they are yellow, and are red only where several crystals cross. This distinguishes them from carotin, for the colors of the solutions are very similar. It melts at 172° . Xanthophyll is relatively stable towards oxygen in dilute sol., but the pulverized substance takes up 36.5 percent of its weight of oxygen, giving a compound, which, precipitated from methyl alcohol by ether, has the formula, $C_{40}H_{56}O_{18}$. Like carotin, it gives a di-iodid, tufts of thin, dark violet, prisms with metallic luster. It is easily decomposed. The bromid, $C_{40}H_{40}Br_{22}$, is also similar to that of carotin. It gives the same color reactions with conc. sulfuric acid and alcoholic hydrochloric acid sol.

Lutein.³¹ As mentioned above, a compound isomeric with xanthophyll has been found in lutein, the coloring matter of egg-yolk. This was first isolated in a pure state by Willstätter and Escher,³² who obtained 4 gm. of very crude pigment from 6000 eggs (110 k.). The yolks were coagulated with alcohol (7 l. to 6 k. of eggs) and the coagulum extracted with acetone (5.4 k. were shaken with 3 l. of acetone and filtered; 2.8 k. of the residue were shaken with 2 l. of acetone for one hour and then washed on a filter with 2 l. of acetone). Phosphatids were removed by shaking the acetone with petroleum ether, washing with water, and mixing the petroleum-ether sirup with two vol. of acetone; the acetone was then removed by washing with water, the petroleum ether concentrated to about 2 l., filtered from cholesterol, diluted to about 6 l. and cooled.

³¹ Although lutein is an animal pigment, its close relationship to xanthophyll warrants its inclusion here.

³² Willstätter and Escher: *Ztschr. f. physiol. Chem.*, 76, 214, 1911-1912.

The lutein that separated was purified by repeated crystallization from methyl alcohol (1 gm. required 1000 cc. for solution) or from carbon disulfid. It formed dark, brownish-yellow, compact prisms with blue surface-luster, melting at 195–6°. It differed from xanthophyll only in its higher melting point, and was called *xanthophyll b* by Willstätter.

Fucoxanthin. Fucoxanthin is the carotinoid characteristic of the *Phaeophyceae*, or brown algae. It differs from the other yellow pigment, in its high oxygen content, having the formula, $C_{40}H_{54}O_6$. Many investigators³³ have had more or less pure solutions of this pigment, but Willstätter and Page³⁴ were the first to obtain a crystalline product.

Fucoxanthin was isolated from the mother liquor of chlorophyll *a* (extracted with 85 percent acetone). Four liters of the extract were treated with 1 l. of a mixture of petroleum ether (30–50°, 3 vol.) and ether (1 vol.) and then with 1.5 l. of water. The ether mixture was then carefully washed free from acetone, concentrated to about ½ l. and shaken with 1 l. of methyl alcohol (saturated with petroleum ether) four times, then twice with ½ l. of alcohol. The xanthophyll was removed by shaking with an equal vol. of a mixture of 5 vol. of petroleum ether and 1 vol. of ether. The fucoxanthin was then transferred to a large vol. of ether, and the ether concentrated to a thick sirup. Fucoxanthin crystallized out upon the addition of low-boiling petroleum ether. The yield from 20 k. of fresh algae was about 2 gm. of a product 85 percent pure.

The use of all reagents containing mineral matter must be avoided, if ash-free preparations are desired. It is also essential that all extracts and solutions be kept from the light and from moisture as much as possible. If the algae are dried previous to the extraction, the yield is very much smaller; and if this dry material is kept for some time before being used, little if any fucoxanthin can be isolated.

The crude product may be recrystallized from methyl alcohol, forming bluish, glistening, brownish-red, long, monoclinic prisms, containing 3 molecules of alcohol. From methyl alcohol or acetone, in the absence of air, it forms dark red six-sided tables containing

³³ Cf. Gaidukow: *Ber. d. deutsch. bot. Gesellschaft.*, 21, 538, 1903. Tswett: *Ibid.*, 24, 234, 1906. Kylin: *Ztschr. f. physiol. Chem.*, 82, 221, 1912.

³⁴ Willstätter and Page: *Ann. d. Chem.*, 404, 237, 1914.

2 molecules of water. These forms are interchangeable. It is obtained free from solvent by precipitation from absolute ether with low-boiling petroleum ether, forming compact needles, melting at $159.5\text{--}160.5^\circ$, depending upon the rate of heating. The ether solution is orange-yellow; the alcoholic sol. more red, with a brownish-yellow tinge; while the carbon di-sulfid sol. is quite red. The pure pigment is stable in an atmosphere of oxygen, but is oxidized in benzene or dilute alcoholic sol., giving a product of approximately the composition, $\text{C}_{40}\text{H}_{54}\text{O}_{16}$.

Fucoxanthin does not show acid properties; it is not extracted from ether by aqueous potassium hydroxid sol. and is not changed by 50 percent hydroxid sol., solid barium hydroxid or metallic sodium. It reacts with alcoholic potash, forming an addition product. This is decomposed by water but gives, instead of fucoxanthin, a product with increased basic properties and a different absorption spectrum.³⁵ The ether sol. gives a deep blue salt with dilute hydrochloric acid sol. This behavior may indicate the existence of a pyrone ring in the fucoxanthin. The reaction with alcoholic potash appears to consist in the decomposition of a part of the pyrone nucleus; the hydroxyl group thus formed would account for the increase in basic properties.³⁶ A characteristic of fucoxanthin is its marked basic properties. The other carotinoids give a deep blue color only with conc. sulfuric acid. Fucoxanthin reacts as a weak base with dilute mineral acids. Thirty percent hydrochloric acid sol. decolorizes the ether sol., itself becoming violet blue in color. With a solution of the acid in dry ether the hydrochlorid, $\text{C}_{40}\text{H}_{54}\text{O}_6 \cdot 4\text{HCl}$, is obtained as blue flakes with a copper luster. When shaken with ether and sodium bicarbonate, a compound is formed with one atom of chlorine, which gives a greenish yellow solution. The iodid, $\text{C}_{40}\text{H}_{54}\text{O}_6\text{I}_4$, forms violet-black, short pointed prisms with a copper luster.

One k. of fresh algae (*Fucus*) contains 0.169 gm. of fucoxanthin, 0.089 gm. of carotin, 0.087 gm. of xanthophyll and 0.503 gm. of chlorophyll *a*.

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³⁵ Xanthophyll is stable towards alcoholic potassium hydroxid.

³⁶ Willstätter and Pummerer: *Ber. d. deutsch. chem. Gesellsch.*, 37, 3740, 1904; 38, 1461, 1905.

PLANT PIGMENTS

Their color and interrelationships

B. HOROWITZ

Introduction. In attempts to explain the action of ammonia on thymol,¹ Prof. Gies and the author were led to review the work of Liebermann on the influence of ammonia upon orcinol.² Liebermann's suggestion that ammonia combines with oxygen of the air to form nitrous acid, and that the latter is the effective agent in the production of pigment, strengthened our view, as already held, that many plant pigments are synthesized in a similar way. Miss Wakeman's study of the pigments in the *Monardas*,³ whereby she came to the conclusion that these are probably oxidation products of thymol, and its isomer, carvacrol, and Wurster's suggestive paper on the rôle of hydrogen peroxid in color formation,⁴ afforded further evidence in support of this idea. During the past year the thymol problem has been studied side by side with an investigation into the chemistry of some plant pigments (the botanical side of which is engaging the attention of Dr. A. B. Stout, of the N. Y. Botanical Garden). As an introduction to a description of these studies, we present herewith a brief review of the theories on color and chemical constitution, as well as an outline of the possible chemical interrelationships of some of the more important plant pigments.

Color and chemical constitution. Perhaps one of the most fascinating chapters in the development of organic chemistry has been the attempt to correlate the chemical constitution of substances with their physical properties. With the rise of synthetic chemistry, and especially as a result of the pioneer work of Graebe, Liebermann and Baeyer in the production of synthetic dyes, color

¹ Gies: *BIOCHEM. BULL.*, 1912, ii, p. 171. Horowitz and Gies: *Ibid.*, 1913, ii, p. 293. Horowitz: Dissertation, Columbia Univ., 1913, pp. 68.

² Liebermann: *Ber. d. d. chem. Gesell.*, 1874, vii, p. 247.

³ Wakeman: *Bulletin of the Univ. of Wisconsin*, No. 448; Science series, 1911, iv, p. 25.

⁴ Wurster: *Ber. d. d. chem. Gesell.*, 1887, xx, p. 2934.

and chemical constitution began to attract attention. Witt's chromogen-chromophore theory, as well as the quinone theory of Armstrong, held absolute sway for many years; and though their usefulness is far from exhausted, the tendency at the present time seems to be to rely less on the influence of the *radical* in the alteration of color, and more on the relationship of color to the *absorption spectra* produced.

As is well known, colored substances exhibit the phenomenon of selective absorption; that is, whenever a body vibrates so as to emit waves of certain definite periods, any waves of these periods falling upon the body will be absorbed. This gives rise to the absorption spectra that have so often been of use in the identification of complex colored compounds. Introduction of radicals into a compound, transforming it from a colorless to a colored substance, with consequent exhibition of absorption in the visible part of the spectrum, may be explained by assuming that the oscillation-frequency has been altered; for example, benzene, though colorless shows absorption bands in the ultra-violet portion. Introduction of the azo group, —N=N— , gives red azo-benzene, with absorption bands in the visible part of the spectrum. What apparently occurs in this case is a change of the short wave-length with its high oscillation-frequency (as found in the ultra-violet region) into a longer wave-length and a consequent slower oscillation-frequency.

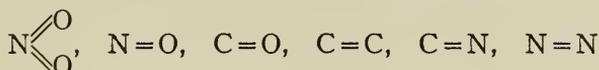
Application of the inductive method in attempts to draw general conclusions has been but partially successful. Even early in the course of these studies it was recognized that unsaturation in a compound is essential to the development of color. Attempts were also made to trace relationships between the molecular weights of compounds and the probable colors produced. This culminated in Nietzski's rule: "The simplest colored substances are in the greenish yellow and yellow, and with increasing molecular weight the color passes to orange, red, violet, blue and green." Like most of the theories in this field, this is at best highly imperfect.

Undoubtedly the most fruitful theory which has so far been advanced connecting color with chemical constitution is that due to Witt.⁵ He considered color to be due to the presence of a "chromophore" group in the molecule. The resulting "chromogen"

⁵ Witt: *Ber. d. d. chem. Gesell.*, 1876, ix, p. 522; 1888, xxi, p. 325.

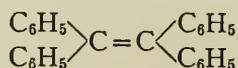
(as the substance containing the "chromophore" group is called) may itself be colored, or yield color by the addition of an "auxochrome" group. For example, benzene itself is colorless; the addition of a "chromophore" group such as $-\text{N}=\text{N}-$, gives the "chromogen," azo-benzene, which is red. On the other hand, the "chromophore," $=\text{C}=\text{O}$, is so weak that benzophenone, $\text{C}_6\text{H}_5-\text{CO}-\text{C}_6\text{H}_5$, is a colorless "chromogen." In neither case, however, is the true coloring matter, or dye, formed, till the "auxochrome" is added. Thus, amino-benzophenone (yellow) and amino-azobenzene, each with the "auxochrome" $-\text{NH}_2$, are true dyestuffs.

The more important "chromophore" groups are

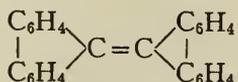


The first four are decidedly weak in their action. An increase of nitro-groups, instead of increasing color—which is usually the case with increase of chromophoric groups—decreases it; thus, nitro-benzene is yellow, but dinitro- and trinitro-benzene are colorless. On the other hand, diphenylethylene, $\text{C}_6\text{H}_5-\text{CH}=\text{CH}-\text{C}_6\text{H}_5$, with a single $\text{C}=\text{C}$ group, is colorless but diphenyl-hexatriene, $\text{C}_6\text{H}_5-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{C}_6\text{H}_5$, is yellow. The same is true of the carbonyl group. One $\text{C}=\text{O}$ (as in the aldehydes, for example) is of no effect; and here even the presence of more than one of these groups will not produce color, if they are separated in the molecule. Acetyl-acetone, $\text{CH}_3-\text{CO}-\text{CH}_2-\text{CO}-\text{CH}_3$, is colorless, but di-acetyl, $\text{CH}_3-\text{CO}-\text{CO}-\text{CH}_3$, is yellow.

The ring structure seems to have a marked influence on the development of color. The nitro-paraffins are colorless, whereas a large number of the aromatic nitro-compounds are colored. Tetraphenyl ethylene,

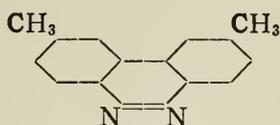


is colorless, but bis-diphenylene ethylene,



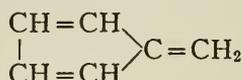
is red. On the other hand, in the azo group, which is among the strongest of the chromophoric groups, ring structure seems to inter-

fers with color formation. Thus, diazomethane, $\text{CH}_2 \begin{matrix} \diagup \text{N} \\ \parallel \\ \diagdown \text{N} \end{matrix}$ is yellow, but tolazone,



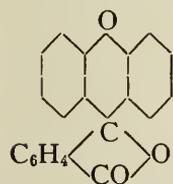
is but slightly colored.

Of special interest is the influence that the *position* of the double bonds may have. Benzene, as has been pointed out, though colorless, shows absorption bands in the ultra-violet part of the spectrum; fulvene,

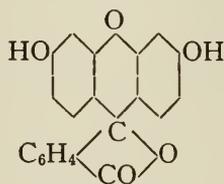


an isomer of benzene, with the *same number* of double bonds, is yellow.

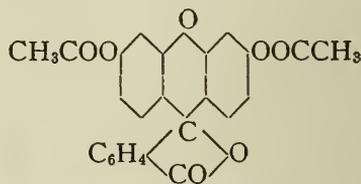
The most important "auxochromes" are the amino and hydroxyl groups, of which the former is the stronger. The color is usually intensified by increasing the number of auxochromes, or, in the case of the amino group, by substituting alkyl and aryl radicals for the hydrogen atoms. On the other hand, acetylating a hydroxyl group inhibits auxochromic action:



Fluoran (chromogen), colorless

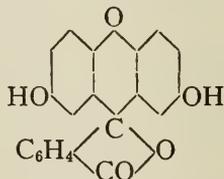


Fluorescein, brown



Diacetyl fluorescein, colorless

That the position of the "auxochrome" group may be of importance is shown by contrasting quinolphthalein,

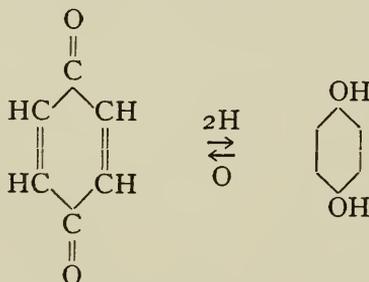


which is colorless, with its isomer, fluorescein.⁶

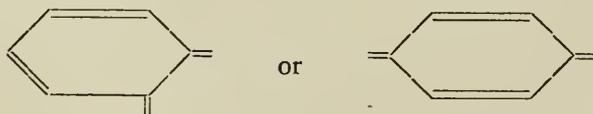
⁶ Hewitt: Chromophores and chromogens, Thorpe's Dict. of Applied Chem., 1912, ii, p. 59.

No relationship seems to have been worked out with reference to the influence of the position of the "auxochrome" relative to that of the "chromophore" group. In some cases when the two are in the ortho position with respect to one another, the intensity of color seems most marked; in others, exactly the reverse is noticed.

Even this brief outline of Witt's theory suffices to show its many shortcomings, though its suggestiveness cannot be questioned. In 1888 Armstrong put forward his quinone theory of color.⁷ In some respects this theory has shown a distinct advance over Witt's conception. Bearing in mind the fact that dyestuffs in general can be reduced to their colorless leuco bases by the addition of hydrogen, Armstrong traced all these compounds to colored quinone, and its reduction product, hydroquinone:



and he came to regard ortho- and para-quinone as the parent substances:



There have been many objections to various phases of this theory. Hartley,⁸ as a result of a careful study of absorption spectra, concludes that color and change of structure do not necessarily go hand in hand, nor is a quinoid nucleus essential in a colored substance. Baeyer's studies of tri-phenyl methyl have led him to similar conclusions, in contradistinction to Gomberg.⁹ Silberrad¹⁰

⁷ Armstrong: *Chem. Soc. Proc.*, 1888, iv, p. 27; 1892, viii, pp. 101, 143, 189, 194.

⁸ Hartley: *Kayser's Handbuch der Spectroscopie*, 1900, iii, p. 170; quoted by Cohen, *Organic Chem.*, 1913, ii, p. 364.

⁹ Gomberg: *Jour. Amer. Chem. Soc.*, 1914, xxxvi, p. 1161. An excellent critical review of this intricate question is given here.

¹⁰ Silberrad: *Jour. Chem. Soc.*, 1906, lxxxix, p. 1787.

has prepared products from melletic and pyromelletic acids, which he cannot under any circumstances regard as quinonic in structure.

It is worthy of note, in this connection, that such simple compounds as quinoneimine, $O=C_6H_4=NH$, and quinonediimine, $HN=C_6H_4=NH$, are colorless. Lately Willstätter¹¹ has succeeded in isolating *colorless* ortho-quinones. To differentiate these from the isomeric orange variety, the superoxid formula,

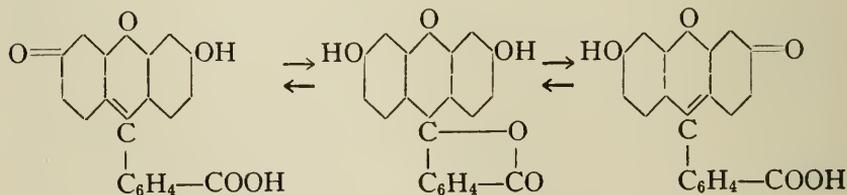


has been assigned to them. This also serves the purpose of emphasizing the absence of the chromophoric group.¹²

Chemical interrelationships.¹³ GENERAL OBSERVATIONS. Until recently the chemistry of plant pigments had not become a subject for systematic research. Thus far only a few standard types of such pigments have been fairly well identified, the great mass of

¹¹ Willstätter: *Ber. d. d. chem. Gesell.*, 1904, xxxvii, p. 4744.

¹² Many interesting problems of a more specific nature were taken up, among others, by Hantzsch (*Ber. d. d. chem. Gesell.*, 1906, xxxix, p. 1073) in his study of the colored nitro-phenol ethers, and his subsequent development of "chromo-isomerism" (isomerism exhibited in change of color); Willstätter (*Ber. d. d. chem. Gesell.*, 1908, xli, p. 1465) and Hewitt (*Trans. Chem. Soc.*, 1907, xci, p. 1251; *Zeit. physik. Chem.*, 1900, xxxiv, p. 1), who has attempted to harmonize his theory of fluorescence with that of color. "Symmetrical compounds," he says, "capable of equal tautomeric displacements in either of two directions, should be those to exhibit the phenomena of fluorescence, for the molecule would swing between the two extreme positions like a pendulum, the energy absorbed of one wave-length being degraded and given out with slower frequency." (Thorpe, *Dic. Applied Chem.*, 1912, ii, p. 59.) Thus, in fluorescein, we have:



This brings fluorescence in relationship to color, the quinonoid structures being the fluorescent substances.

¹³ For a detailed description of individual plant pigments see West: *BIOCHEM. BULL.*, 1915, iv, p. 151 (preceding paper).

material still awaiting further study. It follows from this that no clearly-defined chemical relationships between many of the pigments can be traced. The many theories regarding the origin of plant pigments—chlorophyll in particular—have lost much of their force as a result of a more detailed study of the chemistry of the pigments. Nothing analogous to Baeyer's beautiful conception of sugar synthesis from formaldehyde has been traced for chlorophyll. Perhaps ere long the master mind of Willstätter will have added this achievement to his many others.

From the purely genetic standpoint colors in flowers, especially those due to anthocyanin, may be traced to the following factors:¹⁴ *C*, a chromogen, colored or not colored—possibly a glucosidal flavone.

E, an enzyme (oxidase) which acts on *C* and produces color, giving product *X*.

e, another enzyme, which acts on *X*, giving product *Y*, which differs in color from *X*.

A, an anti-oxidase, which inhibits the action of *E*.

R, reductase, which does the exact opposite to that of the enzyme *E*.

"If a flower only possesses *C* or *E*, then the color will be white or pale yellow, according to the color of the chromogen, if present. If the flower with the factor *C* be crossed with a flower with the factor *E*, then the color of the flowers of the offspring will be red, or a deeper color, if *e* also be present. If either *A* or *R* be present, then there will be no difference in the flowers of the offspring as compared with the parents."

Palladin, in developing his conception of the rôle coloring matters play in respiratory activity of plants, has this to say:¹⁵ "In plants are to be found pro-chromogens which may be regarded as glucosides, or decomposition products of proteins. Enzymes convert the pro-chromogens to chromogens. Oxidases act on the chromogen (in the presence of oxygen) yielding pigments which reductases are capable of reducing again. For example, an oxidase can convert carotin, $C_{40}H_{56}$, into xanthophyll, $C_{40}H_{56}O_2$, a reductase being able to reconvert the latter into the former."

¹⁴ Haas and Hill: Chemistry of plant products, 1913, p. 243.

¹⁵ Haas and Hill: *Ibid.*, 1913, p. 251.

Keeble, Armstrong and Jones¹⁶ suggest that the higher members of a flower-color series owe their origin to the presence, with the lower members, of specific substances which, acting as receivers of oxygen, reduce the pigments characteristic of the lower members of the color series, except oxygen, and then become oxidized to other pigments.

Wheldale¹⁷ classifies pigments other than chlorophyll as follows:

A. *Pigments in solution in cell-sap.*

- (1) Soluble red, purple, blue pigments ("anthocyanin"). Several subclasses.
- (2) Soluble yellow pigments ("xanthin"). Several subclasses.

B. *Pigments associated with specialized protoplasmic bodies—chromoplastids*, the color in this case being usually yellow, orange-yellow, orange or orange-red. Insolubility in water appears to be a constant characteristic of this group.

- (1) Carotin.
- (2) Xanthin.

A more detailed classification is that given by Keeble, Armstrong and Jones, as follows:¹⁸

I. *Plastid pigments.*

- (a) Chlorophyll pigments, containing C, H, O, N.
- (b) Carotin, containing C, H.
- (c) Xanthophyll (oxidized carotin), containing C, H, O.

II. *Sap pigments.*

- (a) Yellow, hydroxy-flavone glucosides or their derivatives, containing C, H, O.
- (b) Red products of the action of oxidase on hydroxy-flavone (glucoside derivatives containing C, H, O).
- (c) Red and brown substances (*e. g.*, the plum) produced by oxidation of phenols in the presence of amino acids, containing C, H, O, N.

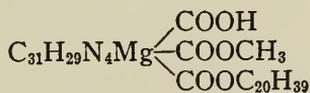
¹⁶ Keeble, Armstrong and Jones: *Proc. Roy. Soc. London* (B), 1914, lxxxvii, p. 113.

¹⁷ Wheldale: *Ibid.*, 1909, lxxxi, p. 44.

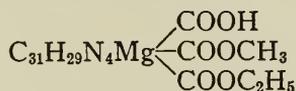
¹⁸ Keeble, Armstrong and Jones: *Ibid.*, 1914, lxxxvii, p. 113.

(d) So-called anthocyan pigments (red and magenta). These may arise in the oxidation of phenols by organic oxygen carriers: contain C, H, O.¹⁹

CHLOROPHYLL. Willstätter²⁰ has shown that the amorphous and the crystalline varieties of chlorophyll are esters of the tri-carboxylic acid, chlorophyllin, $C_{31}H_{29}N_4Mg(COOH)_3$, the amorphous being the methyl-phytyl ester,



and the crystalline, the methyl-ethyl ester,



The fact that carotin and xanthophyll are always associated with chlorophyll has led to attempts to trace the origin of the latter to them. Thus far this has met with no success. However, Monteverde and Lyubimenko,²¹ in studying the transformation of the green leaves of many plants that turn reddish-brown or red in the autumn, have isolated from the red leaves a red pigment (rhodaxanthin) which they suppose is isomeric with xanthophyll.

Most authors seem to be agreed that light is a most important factor in the formation of chlorophyll. Palladin²² and D'Arbamon²³ consider sugar a necessary intermediary, the latter adding starch also. But, even if this were so, the difficulties for the chemist in tracing the synthesis of chlorophyll, with carbohydrate as the starting point, would but begin, now that we know what chlorophyll is chemically.

CAROTIN, $C_{40}H_{56}$, and XANTHOPHYLL, $C_{40}H_{56}O_2$. It has al-

¹⁹ It is interesting to note here that Tswett (*Ber. d. deut. bot. Gesell.*, 1906, xxiv, p. 326; 1907, xxv, p. 137), from studies in absorption spectrum analysis, concluded that there are at least seven different coloring-matters in leaf-pigment.

²⁰ See West's comprehensive review of Willstätter's book on chlorophyll: *BIOCHEM. BULL.*, 1914, iii, p. 229.

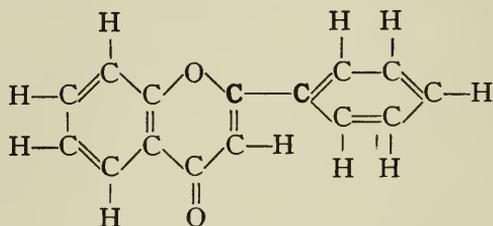
²¹ Monteverde and Lyubimenko: *Bull. Acad. Sci. St. Petersburg*, 1913, p. 1007; *Chem. Abstracts*, 1914, viii, p. 728.

²² Palladin: *Ber. d.d. bot. Gesell.*, 1902, xx, p. 224.

²³ D'Arbamon: *Ann. Sci. Nat. Bot.*, 1909, ix, p. 197.

ready been stated that carotin can be transformed into xanthophyll by oxidation. It is therefore highly probable that the origin of the latter may be attributed to this action. But how and under what conditions, does carotin arise? Here again no answer can as yet be given.

FLAVONES. The mother substance of the yellow, water-soluble pigments, flavone, has the constitution indicated by the formula,



Most of the flavones are readily synthesized from phenols and carboxylic acids. On fusion with alkali they usually yield phloroglucinol and protocatechuic acid, and sometimes resorcinol and hydroxybenzoic acid. These compounds may, therefore, be looked upon as giving rise to the flavone coloring matters.

ANTHOCYANINS. The red and blue coloring matters that can be extracted from leaves, flowers, and fruit by means of water are commonly spoken of as anthocyanins or anthocyanins. No good classification of these substances has as yet been suggested. Willstätter, who has recently begun to investigate them, and whose work promises to shed much light on the subject, in a recent study of the anthocyan of corn flower (cyanin), has found that it hydrolyzes into two molecules of glucose and one molecule of cyanidin ($C_{15}H_{11}O_6Cl$).²⁴

With regard to the mode of formation of these anthocyanins much of interest has been suggested. Miss Wheldale,²⁵ by cross-breeding yellow and white forms, obtained anthocyanin products. As the yellow forms were flavone in nature, and the white contained oxidases, Miss Wheldale drew the conclusion that anthocyanins are oxidation products of flavones. Since the flavones are known to

²⁴ Willstätter: *Sitzb. preuss. Akad. Wissenschaften*, 1914, xii, p. 402. See also Willstätter and Everest, *Ann.*, 1914, cccci, p. 189.

²⁵ Wheldale: *Proc. Cambridge Phil. Soc.*, 1909, xv, p. 137; *Proc. Roy. Soc.*, 1909, B, lxxxii, p. 44; *Biochem. Jour.*, 1913, vii, p. 87.

occur as glucosides in many plants, the following scheme of reaction was suggested:²⁶

(1) Glucoside + water \rightleftharpoons flavone + sugar.

(2) X (flavone) + oxygen \rightarrow anthocyanin.

In addition to oxidation there might be condensation of the flavone molecules. Reaction (1) may be controlled by a glucose-splitting enzyme, and (2) may be due to an oxidase. Many authors have shown that oxygen or oxidase plays an important part in anthocyanin formation.²⁷

Acid turns anthocyanins red; alkali, blue. This characteristic feature of these pigments naturally suggested that the red modification behaves like a weak acid, and the blue like a weak alkali. The fact that an excess of alkali gives a green instead of blue color has been explained by the assumption that anthocyanin is a bi-basic acid.

Attempts to trace some relationship between anthocyanin and chlorophyll have not been wanting. Thus, it had been stated that leaves containing anthocyanin have relatively less chlorophyll than those which do not contain anthocyanin.²⁸ Macaire's hypothesis that chlorophyll is transformed into anthocyanin held sway for many years, till Mohl disproved it. Mulder was of the opinion that the decomposition of chlorophyll gave rise both to blue and yellow pigments.²⁹ None of these statements has been substantiated.

A close chemical relationship between anthocyanins and flavones has been shown by Willstätter.³⁰ As has been stated Willstätter has found that the anthocyan of corn flower can be hydrolyzed into glucose and cyanidin. Now, if quercetin, a hydroxy-derivative of flavone, is dissolved in alcohol, made strongly acid with hydrochloric acid, and reduced at 35° with Mg-Hg, a small quantity of cyanidin is formed. The solution can be concentrated and the separated cyanidin and quercetin filtered off, dissolved in alcohol, and the cyanidin precipitated with ether:

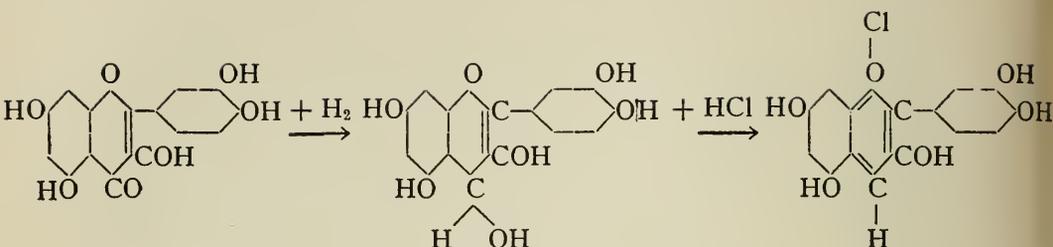
²⁶ Wheldale: *Proc. Roy. Soc. (B)*, 1914, lxxxvii, p. 301.

²⁷ Malvezin: *Compt. rend.*, 1908, cxlvii, p. 348. Molliard: *Ibid.*, 1909, cxlviii, p. 573. Combes: *Ibid.*, 1910, cl, pp. 1186, 1532. Keeble and Armstrong: *Jour. of Genet.*, 1912, ii, p. 277. See also Czapek, *Biochemie der Pflanzen*, 1913, i, p. 591.

²⁸ Haas and Hill: *Chem. of plant products*, 1913, p. 244.

²⁹ See Czapek: *Biochemie der Pflanzen*, 1913, i, p. 586.

³⁰ Willstätter and Mallison: *Sitz. preuss. Akad. Wiss.*, 1914, xii, p. 769.



This is in direct contradiction to Miss Wheldale's findings. Here the change from a flavone to an anthocyanin product involves reduction, whereas Miss Wheldale regarded the process as one of oxidation.

The exact influence of light in the formation of anthocyanin pigments has yet to be settled.³¹ Ewart³² has shown that, in aquatic plants at least (such as *Elodea canadensis*), the red color does not appear if the plant is grown in diffuse sunlight. Overton³³ names temperature as an additional factor: a low temperature, but one above freezing-point, favors the formation of the pigment. This explains the prevalence of red color in alpine plants.

That anthocyanin formation is dependent upon the presence of sugar is suggested by Overton's interesting experiments.³⁴ He found that the pigment formation could be artificially induced by immersing the cut leaves of many plants in a 2-3 percent sugar solution. This finding was later confirmed and extended by Combes³⁵ and Rose.³⁶ The fact that anthocyanins are commonly found with tannin-like substances has led Wigand to regard the two as closely allied. Anthocyanins give the iron reaction and, like the tannins, are precipitated by caffein and antipyrin.³⁷

Biochemical Laboratory of Columbia University,
College of Physicians and Surgeons, New York.

³¹ Fischer: *Flora*, 1908, xcvi, p. 380. Chartier and Colin: *Rev. gen. Botan.*, 1911, xxiii, p. 264. Landel: *Compt. rend.*, 1893, cxvii, p. 314.

³² Ewart: *Ann. Bot.*, 1897, xi, p. 461.

³³ Overton: *Nature*, 1899, lix, p. 296.

³⁴ Overton: *Jahr. wiss. Botan.*, 1899, xxxiii, p. 171.

³⁵ Combes: *Compt. rend.*, 1909, clxviii, p. 790.

³⁶ Rose: *Ibid.*, 1913, clviii, p. 955.

³⁷ See Czapek: *Biochemie der Pflanzen*, 1913, i, p. 587. For the chemistry of tannins, especially with reference to an attempted synthetic production, see Fischer: *Jour. Amer. Chem. Soc.*, 1914, xxxvi, p. 1187.

FURTHER COMMENT ON MUSCULAR WORK AND RESPIRATORY QUOTIENT

In my note on "Muscular Work and Respiratory Quotient," in the last number of the *BIOCHEMICAL BULLETIN*,¹ it was erroneously stated that Benedict and Cathcart, in their monograph on this subject, did not mention the rate at which the air was circulated in the apparatus used by them for measuring the gaseous metabolism of the bicycle rider. I find now, however, an allusion to this matter in the text, according to which a tremendous ventilation of 85 liters per minute was maintained during a work experiment, and I am glad to correct the error committed in my note.

In discussing the shortcomings of Benedict and Cathcart's technic, I assumed that probably 600 liters of air passed through the sulfuric acid in the course of a work experiment. Since these experiments usually lasted ten minutes, a ventilation of 60 liters per minute, or one liter per second, was postulated in my argument. Considering the peculiar arrangement of the sulfuric acid absorption system in their apparatus, it must have been impossible to free such a rapid current of saturated air of all its moisture. Now, according to Benedict and Cathcart's own statement, 85 liters of air instead of 60, as I had assumed, were actually passed through the sulfuric acid wash bottle every minute, or practically one and a half liters per second.

It is obvious that while I erred in saying that no information is given in the monograph regarding the rate of ventilation, my argument is *strengthened* by the fact alluded to above and which was overlooked in the preparation of my first note on this subject.

SERGIUS MORGULIS

*College of Physicians and Surgeons,
Columbia University, New York.*

¹ Morgulis: *BIOCHEMICAL BULLETIN*, 1914, iii, p. 435.

THE BIOCHEMICAL SOCIETY, ENGLAND

Scientific programs

R. H. A. PLIMMER, SECRETARY

October 16.¹ PHYSIOL. LAB., UNIV. OF LONDON, SOUTH KENSINGTON, S. W. (5.30 P. M.).

J. C. Drummond and C. Funk: Chemical investigation of some rice-polishings fractions.

S. S. Zilva (introduced by A. Harden): The rate of destruction by heat of the peroxydase of milk.

A. Harden and R. Robison: A new phosphoric ester obtained by the aid of yeast juice.

S. Walpole: Demonstration of cataphoresis apparatus—Hermann's phenomenon.

H. H. Dale and G. Barger: Liver-nitrogen in anaphylaxis.

J. A. Gardner: Respiratory exchange of fish under low oxygen tension.

December 8.² LISTER INST., CHELSEA GARDENS, LONDON, S. W. (5.30 P. M.). Demonstrations of "micro-methods" of analysis.

G. Barger: Determination of molecular weights.

H. Maclean: Estimation of glucose in blood.

O. Rosenheim: Van Slyke's method of estimating amino nitrogen.

C. Funk and J. C. Drummond: (a) Pregl's method of analysis of carbon, hydrogen and nitrogen. (b) Kjeldahl's method of estimating nitrogen (Bang).

E. C. Grey: Analysis of aliphatic compounds by moist combustion.

March 11.³ (Annual general meeting). MEDICAL LECTURE

¹ The Society did not meet in August or September. See BIOCHEMICAL BULLETIN, 1914, iii, p. 452.

² The Society did not meet in November.

³ The Society did not meet in January or February. The next meeting will be a joint session, on May 5, with the Society of Public Analysts. It will be

THEATRE, St. Bartholomew's Hospital and Coll., London, E. C. (5.30 P. M.).

S. Walpole: Counter diffusion in aqueous solutions.

B. Moore: Photosynthesis by inorganic catalysts.

B. Moore: Forms of growth or deposit arising in metastable colloidal solutions.

G. Graham and W. H. Hurtley: The effect of the vegetable-egg diet on severe diabetes.

R. L. Mackenzie Wallis: The estimation of the diastatic activity of the urine.

G. Winfield: The fate of fatty acids in the survival processes of muscle.

W. B. Bottomley: The formation of humus from organic substances.

At this meeting the following officers were elected: Hon. Treasurer, *J. A. Gardner*; Hon. Secretary, *R. H. A. Plimmer*; Ordinary members of the Committee, *W. A. Davis*, *T. A. Henry*, and *H. M. Vernon*, to succeed *F. G. Hopkins*, *E. J. Russell* and *J. S. Ford*.

University College, London.

devoted to a discussion of "methods adopted in the estimation of the nitrogenous constituents of extracts derived from albuminous substances, such as meat extracts and similar products, with special reference to the interpretation of the results."

THE FEDERATION OF AMERICAN SOCIETIES FOR
EXPERIMENTAL BIOLOGY*

Peace resolution

The following resolution was unanimously adopted at the annual meeting held in Saint Louis, on the twenty-eighth of December, One thousand, nine hundred and fourteen:

WHEREAS, Various of the European nations with which many of our members are related by birth, descent, or intellectual friendship, are now at war;

Resolved, That we extend to the scientific men within these nations the hope of an early and enduring peace, which will leave the nations with no permanent cause of rancor towards each other, and which will insure to each the glories of scientific and humanitarian achievement in accordance with its own conception of these ideals.

THE PHYSIOLOGICAL SOCIETY,

Walter B. Cannon, *President*;

THE SOCIETY OF BIOLOGICAL CHEMISTS,

Graham Lusk, *President*;

THE SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS,

Torald Sollman, *President*;

THE SOCIETY FOR EXPERIMENTAL PATHOLOGY,

Richard M. Pearce, *President*;

PHILIP A. SHAFFER,
Secretary of the Federation.

*The above is a copy of a formal statement that was sent to every member of the Federation.—(Ed.)

PROCEEDINGS OF THE SECOND ANNUAL MEET-
ING OF THE FEDERATION OF AMERICAN
SOCIETIES FOR EXPERIMENTAL
BIOLOGY, IN ST. LOUIS,
DEC. 28-30, 1914

PAUL E. HOWE

PREPARED FROM REPORTS BY THE SECRETARIES OF THE CONSTITUENT SOCIETIES,

A. J. CARLSON, P. A. SHAFFER, JOHN AUER AND G. H. WHIPPLE

CONTENTS. (I) Federation of Amer. Soc. for Exp. Biol.: *P. A. Shaffer*, Sec'y of the Exec. Commit. of the Federation, 177; (II) Amer. Physiol. Soc.: *A. J. Carlson*, Sec'y, 180; (III) Amer. Soc. of Biol. Chemists; *P. A. Shaffer*, Sec'y, 182; (IV) Amer. Soc. for Pharmacol. and Exp. Therap.: *John Auer*, Sec'y, 185; (V) Amer. Soc. for Exp. Pathol.: *G. H. Whipple*, Sec'y, 187; (Addendum) Amer. Assoc. of Anatomists: *Charles R. Stockard*, Sec'y, 188.

I. FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL
BIOLOGY:

SECOND ANNUAL MEETING

P. A. Shaffer,

Secretary of the Executive Committee for 1914

The second annual meeting of the Federation, comprising the Amer. Physiol. Soc., the Amer. Soc. of Biol. Chemists, the Amer. Soc. for Pharmacol. and Exp. Therapeutics, and the Amer. Soc. for Exp. Pathol., was held at St. Louis, Dec. 28-30, 1914, in the laboratories of the Washington Univ. Med. School.

Dinners and smokers. This part of the program was inaugurated by a dinner given by the Local Commit., on Sunday evening, Dec. 27, to the officers and councils of the constituent societies of the Federation and of the Anatom. Soc.

The customary and universally satisfactory informal subscription dinners and smokers were held on the evenings of Dec. 28-30; the first two at the Hotel Jefferson, the last one at the Hotel War-

wick. Perhaps the most enjoyable of these was the first, on Dec. 28. On this occasion a number of excellent speeches were delivered, the speakers being the guests of the evening, Mr. Brookings, and Drs. Graham Lusk, J. George Adami and G. Carl Huber.

Scientific program. Three joint sessions of the Federation were held, at which the following papers were presented.

FIRST SESSION. MONDAY, DEC. 28, 9.00 a. m. PRESIDING OFFICER: *President of the Biochem. Soc., and Chairman of the Exec. Commit. for 1914, Graham Lusk.* Memorial addresses: S. Weir Mitchell, by E. T. Reichert (read by W. B. Cannon); Charles S. Minot, by Frederic S. Lee.

W. B. Cannon, C. A. Binger and R. Litz: Experimental hyperthyroidism.—*David Marine:* Further observations on the etiology of goitre in fish.—*H. R. Basinger and A. L. Tatum:* Studies on experimental cretinism.—*G. W. Crile, F. W. Hitchings and J. B. Austin:* A research into the function of the thyroid.—*S. Simpson and R. L. Hill:* The effect of repeated injections of pituitrin on milk secretion.—*W. L. Gaines:* The action of pituitrin on the mammary gland.—*F. P. Knowlton and A. C. Silverman:* On the mechanism of pituitrous diuresis.—*George B. Roth:* The several factors involved in the standardization of pituitary extracts.

SECOND SESSION. TUESDAY, DEC. 29, 2 p. m. PRESIDING OFFICER: *President of the Pharmacol. Soc., Torald Sollman. J. R. Murlin and B. Kramer:* The influence of sodium carbonate on the glycosuria, hyperglycemia and the respiratory metabolism of depancreatized dogs.—*I. S. Kleiner and S. J. Meltzer:* The influence of depancreatization upon the state of glycemia after intravenous injections of dextrose in dogs.—*J. J. R. Macleod:* The possibility that some of the hepatic glycogen may become converted into other substances than dextrose.—*R. T. Woodyatt:* Narcotics in phlorhizin diabetes.—*R. S. Hoskins:* Adrenal deficiency.—*H. McGuigan:* Hypoglycemia.—*J. Auer and F. L. Gates:* Some effects of adrenalin when injected into the respiratory tract.—*G. W. Crile, F. W. Hitchings and J. B. Austin:* The relation of the adrenals to the brain.—*A. B. Macallum and J. B. Collip:* Further observations of the origin of hydrochloric acid in the stomach.—*C. C. Fowler, M. E. Rehfuess and P. B. Hawk:* The effect of various fluids and

cereals on gastric secretion.—*R. W. Keeton* and *F. C. Koch*: The distribution of gastrin in the body.—*F. F. Rogers* and *L. L. Hardt*: The relation of the digestion intractions to the hunger contractions of the stomach (dog, man).

THIRD SESSION. WEDNESDAY, DEC. 30, 9.00 a. m. PRESIDING OFFICER: *President of the Biochem. Soc., and Chairman of the Exec. Commit. for 1914, Graham Lusk*. *F. D. Zeman, J. Kohn* and *P. E. Howe*: Recuperation: Nitrogen metabolism of a man when ingesting successively a non-protein and a normal diet after a seven-day fast.—*H. C. Bradley*: Some studies in autolysis.—*H. McGuigan* and *C. L. v. Hess*: The diastase of the blood.—*W. E. Burge*: The rate of oxidation of enzymes and their corresponding pro-enzymes.—*C. Voegtlin*: The harmful effect of an exclusive vegetable diet.—*C. L. Alsberg* and *C. S. Smith*: The effect of long-continued feeding of saponin from the bark of *Guaiacum officinale*.—*E. L. Opie* and *L. B. Alford*: Fat infiltration of the liver and kidney induced by diet.—*V. H. Mottram*: On the nature of the hepatic fatty infiltration in late pregnancy and early lactation.—*F. B. Kingsbury* and *E. T. Bell*: The synthesis of hippuric acid in experimental tartrate nephritis in the rabbit.

DEMONSTRATIONS. *C. Brooks* and *A. B. Luckhardt*: Blood-pressure method.—*J. Erlanger* and *W. E. Garrey*: Demonstration of a point-to-point method for analyzing induction shocks by means of the string galvanometer.—*B. M. Patten*: A device for projecting a small spot of light suitable for exploring photosensitive areas.—*S. Amberg* and *D. McClure*: Demonstration of the effect of sodium iodoxy-benzoate on inflammation caused by mustard oil.—*Worth Hale*: An arrangement of the Porter clock to give three-time intervals at the same time.—*F. L. Gates*: A portable respiratory machine furnishing continuous, intermittent and remittent streams of air.—*P. A. Shaffer*: The determination of blood sugar.

Executive proceedings. The resolution printed on page 176 of this volume was unanimously adopted.

EXECUTIVE COMMITTEE FOR 1915. Chairman—*Torald Sollmann*; Secretary—*John Auer* (Pharmacol. Soc.); *W. B. Cannon*, *C. W. Greene* (Physiol. Soc.); *Walter Jones*, *P. A. Shaffer* (Biochem. Soc.); *Theobald Smith*, *Peyton Rous* (Pathol. Soc.).

NEXT MEETING. The next meeting of the Federation will be held, 1915, in Boston, at the Harvard Medical School.

II. AMERICAN PHYSIOLOGICAL SOCIETY:
TWENTY SEVENTH ANNUAL MEETING

A. J. Carlson, Secretary

The twenty-seventh annual meeting of the Physiol. Soc. was held in the Physiol. laboratories of the Washington Univ. Med. Sch., St. Louis, Mo., Dec. 28-31, 1914. Fifty-six of the Society's 208 members were present. Five scientific sessions were held, three of these being joint meetings with the other societies of the Federation. At the two independent meetings the following papers were presented.

Scientific program. FIRST SESSION. MONDAY, DEC. 28, 2.00 p. m. *F. S. Lee* and *D. J. Edwards*: The action of certain atmospheric conditions on blood-pressure and heart-rate.—*C. H. Dallwig*, *A. C. Kolls* and *A. S. Loevenhart*: The relation between the erythrocytes and the hemoglobin to the oxygen of the respired air.—*J. A. E. Eyster* and *W. J. Meek*: The path of conduction for the cardiac impulse between the sino-auricular and the aurico-ventricular nodes.—*C. Brooks* and *A. B. Luckhardt*: An experimental and critical study of blood-pressure methods.—*F. C. Becht* and *H. McGuigan*: Mechanical factors in the flow of cerebro-spinal fluid.—*J. F. McClendon*: Oxidation in the erythrocytes of the goose (with note on a baro-thermostat).—*Katherine R. Drinker* and *C. K. Drinker*: The effect of rapid and progressive hemorrhage upon the factors of coagulation.—*S. Simpson* and *A. T. Rasmussen*: The effect of parathyroidectomy on blood-coagulation time in the dog.—*F. C. McLean*: On the concentration of sodium chlorid in the serum and its relation to the rate of excretion in normal and diabetic men.—*W. H. Spencer*, *M. E. Rehfuess* and *P. B. Hawk*: Does regurgitation regulate the acidity of gastric juice?

SECOND SESSION. TUESDAY, DEC. 29, 9.00 a. m. *T. S. Githens* and *S. J. Meltzer*: *Apnea vera* without previous excess of respiration, and its dependence upon the vagus nerves.—*M. L. Fleisher* and *Leo Loeb*: The lytic action of tissues on blood coagulum.—*Ida H. Hyde*: The influence of light on the development of vorticella.

—*S. Tashiro*: The metabolism of the resting nerve and its correlation to the direction and rate of the nerve impulse.—*R. G. Pearce*: Renal secretory nerve fibres.—*A. L. Beifeld, H. Wheelon* and *C. R. Lovelette*: The effect of pancreas extract on sympathetic irritability.—*B. H. Schlomovitz, J. A. E. Eyster* and *W. J. Meek*: Distribution of chromotropic vagus fibres within the sinoauricular node.—*Ida H. Hyde*: The relation of the nervous system to a tunicate larva.—*J. F. McClendon*: Some experiments on the oxidizing power of oxyhemoglobin.

PAPERS READ BY TITLE. *E. G. Martin* and *P. G. Stiles*: Some characteristics of vasomotor reflexes.—*M. Dresbach*: Experiments on transplantation of the pancreas.—*W. J. Meek* and *J. A. E. Eyster*: The action of adrenalin in minimal doses.—*E. G. Martin*: The validity of inductorium calibrations.—*A. J. Carlson*: The alleged action of the bitter tonics on the secretion of gastric juice in man and dog.—*A. J. Carlson* and *H. Ginsburg*: Blood transfusion in pancreatic diabetes.—*A. J. Carlson*: On the secretion of gastric juice in man.—*J. F. McClendon*: Increase of permeability of the frog egg at the beginning of development, as determined with the nephelometer.

In addition to eight papers that had been placed on the program to be read by title, sixteen communications which were to have been orally reported were read by title only, because the authors were absent from the meeting. The failure of the authors of these sixteen papers to appear seriously marred the scientific program. The Sec'y hopes that this meeting will be the high-water mark of the bad habit of reporting papers to be read without going to the meeting to present them. In cases of unavoidable absence through sickness, the Sec'y should be notified, so that readjustment may be made even after the program is in print. As for those who asked to be placed on the program and then chose to stay away from the meeting, the Sec'y feels that the annual meetings of the society are too important to be made the subject of practical jokes of that type.

Executive proceedings. CONSTITUTION. Some important changes in the constitution were adopted. The importance of research as the qualification for election to membership in the society was more explicitly emphasized. Voting by mail or proxy was abolished.

AMER. JOUR. OF PHYSIOL. The management of the *American Journal of Physiology*, owned and published by the Society, was entrusted to the Council. The Council was enlarged from five to seven members.

In recognition of Dr. W. T. Porter's great service to physiology, in founding the *Amer. Jour. of Physiol.* and successfully publishing it for many years, the Council was entrusted to arrange for the dedication, to Dr. Porter, of a volume of the *Journal*. (See p. 245.)

NEW MEMBERS: *A. Arkin*, Univ. West Va.; *A. T. Cameron*, Univ. Manitoba; *P. M. Dawson*, Univ. Wis.; *C. M. Gruber*, *E. B. Krumbhaar*, Univ. Pa.; *E. N. Harvey*, Princeton Univ.; *H. L. Higgins*, Nutrition Lab., Carnegie Inst.; *Jessie L. King*, Goucher Coll.; *F. C. McLean*, Rockefeller Inst.; *S. Morgulis*, *E. L. Scott*, Columbia Univ.; *G. B. Roth*, Hygienic Lab., Wash.

OFFICERS-ELECT: President—*W. B. Cannon*; Secretary—*C. W. Greene*; Treasurer—*J. Erlanger*; Additional members of the Council—*W. H. Howell*, *J. J. R. Macleod*, *W. E. Garrey*, *W. J. Meek*.

COMMENT. Despite the unusual defaults in the matter of the scientific program, and the presence of only a few members from the Atlantic seaboard, the meeting was a success, due largely to the considerate efforts and the generous hospitality of the Local Committee. The opportunity to inspect the new laboratories and hospitals of Washington Univ. Med. School itself justified the trip to St. Louis. It appears that this school has actually made an advance beyond the "stone age" of American universities in general. In material equipment for medical research and teaching, Washington Univ. Med. Sch. is second to none, if not superior to all other medical schools in this country.

III. AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS:

NINTH ANNUAL MEETING

P. A. Shaffer, Secretary

The ninth annual meeting of the Biochem. Soc. was held at St. Louis, on Dec. 28-30, 1914, in the laboratories of the Washington Univ. Med. Sch. Three joint sessions were held with the other

societies composing the Federation, in addition to two sessions conducted independently. The following communications were presented at the independent sessions.

Scientific program. FIRST SESSION. MONDAY, DEC. 28, 2.00 p. m. *Graham Lusk*: Presidential address, The influence of food on metabolism.—*S. R. Benedict* and *E. Osterberg*: The retention of parenterally introduced creatin under various nutritive conditions in the dog.—*Otto Folin* and *W. Denis*: The occurrence of creatin in urine.—*F. D. Zeman* and *P. E. Howe*: The excretion of creatin during fasting.—*J. L. Morris*: The determination of creatin and creatinin in urine; and the occurrence of creatin.—*W. C. Rose*: The influence of protein feeding on the elimination of creatin in starvation.—*P. A. Kober*: The nephelometric estimation of purin bases, including uric acid, in blood and urine.—*W. H. Welker* and *Grover Tracy*: The use of aluminium hydroxid in connection with nitrogen partition in urinary analysis.—*H. R. Fishbach* and *P. B. Hawk*: The fecal bacteria output as influenced by dietary alterations.—*N. Hendrickson*, *E. L. Connolly*, *B. M. Hendricks* and *M. E. Pennington*: The dextrose content of the egg of the common fowl.—*H. J. Corper*: A method for determining and comparing the local toxicity of chemical compounds.—*E. A. Graham*: The mechanism of the toxicity of halogen narcotics.

SECOND SESSION. TUESDAY, DEC. 29, 9.00 a. m. *Olaf Bergheim*: Some influences affecting the action of phospho-nuclease.—*H. H. Bunzel*: Biological oxidizability and chemical constitution (II).—*H. I. Mattill* and *H. A. Mattill*: Digestive processes in *Limulus*.—*R. E. Swain*: The action of alkaline hydrolytic agents on allantoin.—*Arno Viehoever*, *C. O. Johns* and *C. L. Alsberg*: Cyanogenesis in plants: (I) Studies on *Sieglingia sesleroides*.—*R. T. Woodyatt*: Experiments with *d-l*-glyceric aldehyde.—*J. J. R. Macleod* and *R. G. Pearce*: The level of sugar in the blood flowing from the liver under laboratory conditions.—*F. S. Lee* and *E. L. Scott*: The action of certain atmospheric conditions on muscular work and blood-sugar.—*P. A. Shaffer* and *R. S. Hubbard*: The level of blood-sugar in the dog.—*C. C. Fowler* and *P. B. Hawk*: Sulfur partition as influenced by water drinking.—*E. C. Kendall*: A method for the decomposition of the proteins of the thyroid with a description of

certain constituents.—*F. D. Zeman, Jerome Kohn and P. E. Howe*: Variations in factors associated with acidity of human urine during a seven-day fast and during subsequent non-protein and normal feeding periods.

PAPERS READ BY TITLE. *Jacob Rosenbloom*: The effect of intravenous injections of radium on the urinary nitrogen and sulfur partition.—*Jacob Rosenbloom*: The effect of external application of radium on the metabolism of a cancer patient.—*P. H. Mitchell*: Carbohydrate metabolism in the oyster.—*Amos W. Peters*: Studies on the pathology of the feeble-minded: (I) The glycosuric reaction and its relation to their pathology.—*G. W. Raiziss and H. Dubin*: A method for the determination of benzoic acid in urine.—*G. W. Raiziss and H. Dubin*: The synthesis of hippuric acid in the animal body.

Executive proceedings. NEW MEMBERS: *Olaf Bergeim*, Jefferson Med. Coll.; *Alex. T. Cameron*, Univ. Manitoba; *G. H. A. Clowes*, Gratwick Lab., Buffalo, N. Y.; *B. M. Duggar*, Missouri Botan. Garden; *Cyrus H. Fiske*, Harvard Med. Sch.; *R. A. Hall*, Univ. Minn.; *C. G. Inrie*, Univ. Toronto; *Benjamin Kramer*, State Univ. Iowa; *A. Bruce Macallum, Jr.*, Univ. Toronto; *J. F. McClen-don*, Univ. Minn.; *J. Lucien Morris*, Washington Univ. Med. Sch.; *Max Morse*, Univ. Wis.; *V. H. Mottram*, McGill Univ.; *C. F. Nel-son*, Univ. Kansas; *E. L. Ross*, Northwestern Univ. Med. Sch.; *E. C. Shorey*, U. S. Dep't of Agric.

OFFICERS-ELECT: President—*Walter Jones*; Vice-president—*Carl L. Alsberg*; Secretary—*P. A. Shaffer*; Treasurer—*D. D. Van Slyke*; Additional members of the Council—*Otto Folin, Graham Lusk, L. B. Mendel*; Nominating Commit.—*J. J. Abel, S. R. Bened-ict, H. D. Dakin, P. B. Hawk, J. J. R. Macleod, E. V. McCollum V. C. Myers, T. B. Osborne, A. N. Richards*.

ATTENDANCE. *J. G. Adami, S. Amberg, L. Baumann, H. C. Bradley, H. J. Corper, C. H. Fiske, W. E. Garrey, A. D. Hirsch-felder, R. A. Hall, P. E. Howe, E. C. Kendall, B. Kramer, A. S. Loevenhart, G. Lusk, J. J. R. Macleod, V. H. Mottram, H. Mc-Guigan, J. L. Morris, C. H. Neilson, E. W. Rockwood, P. A. Shaffer, T. Sollmann, C. Voegtlin, H. G. Wells, R. T. Woodyatt*.

IV. AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS:

SIXTH ANNUAL MEETING

John Auer, Secretary.

The sixth annual meeting of the Pharmacol. Soc. was held in St. Louis, at the Washington Univ. Med. Sch., on December 27-30, 1914. There were five scientific sessions, three of them being joint meetings with the other members of the Federation. At the two independent sessions the following papers were read.

Scientific program. FIRST SESSION. MONDAY, DEC. 28, 2.00 p. m. *S. Amberg* and *H. F. Helmholtz*: The fatal dose of various substances on intravenous injection in the guinea-pig.—*G. W. Crile*: Experimental and clinical research into alkalescence, acidity and anesthesia.—*P. J. Hanzlik*: Effects of chelidinin on surviving organs.—*T. Sollmann*, *W. L. Mendelhall* and *J. L. Stingle*: The effect of temperature on the response of frogs to ouabain.—*E. D. Brown*: Artificial cerebral circulation after circulatory isolation of the mammalian brain.—*Worth Hale*: The uterine action of quinidin, cinchonin and cinchonidin.—*C. D. Edmunds*: Some vasomotor reactions in the liver.—*T. S. Githens* and *S. J. Meltzer*: Distribution of solutions in cardiectomized frogs with destroyed or inactive lymph hearts.—*F. L. Gates* and *S. J. Meltzer*: The influence of intra-intestinal administration of magnesium sulfate upon the production of hyalin casts in dogs.

SECOND SESSION. TUESDAY, DEC. 29, 9.00 a. m. *W. deB. MacNider*: A study of the relative importance of the vascular mechanism of the kidney and of the epithelial element of the kidney in determining the efficiency of various diuretics.—*H. B. Myers*: Cross-tolerance of drugs.—*H. B. Myers* and *G. B. Wallace*: Vascular reactions in poisoning from diphtheria toxin.—*A. D. Hirschfelder*: The action of digitalis in experimental auricular fibrillation.—*A. D. Hirschfelder*: The effects of drugs upon the circulation in the *Pia mater* and the retinal vessels.—*Clyde Brooks* and *J. D. Heard*: The action of camphor on the circulation.—*Don R. Joseph*: The effect of carbon dioxide upon the convulsant action of acid fuchsin in frogs.—*Carl Voegtlin*: The mechanism of the toxic action of heavy

metals on the isolated heart.—*C. W. Greene, L. R. Boutwell and J. O. Peeler*: An analysis of the action of digitalin on the cardiac inhibitory centre and on the cardiac muscle.—*W. H. Schultz*: A comparative study of the influence of the solvent upon the toxicity of thymol.—*W. H. Schultz*: The reaction of hookworm larvae to certain chemicals.—*A. E. Cohn*: A further observation on the "T-wave" when digitalis is given.

Executive proceedings. NEW MEMBERS: *F. C. Becht*, Univ. Chicago; *W. H. Brown, F. L. Gates*, Rockefeller Inst.

OFFICERS-ELECT: President—*Torald Sollmann*; Secretary—*John Auer*; Treasurer—*Wm. deB. MacNider*; Additional members of the Council—*Worth Hale, D. E. Jackson*. Membership Commit.—*S. J. Meltzer* (term expires 1917).

General comment. Among the topics discussed during the business meetings, was one especially which is of general interest. Several members expressed marked dissatisfaction with the present arrangement of holding the annual meetings during the Christmas holidays. They suggested that practically any other time would be better. Their arguments, briefly, were as follows: The Christmas sessions always break the holidays as a family festival for members who live at some distance from the place of annual meeting; it has not been uncommon for members to spend Christmas day on the train. Secondly, if the meetings were held in June or July,¹ more time would be available for the completion of work started at the beginning of the academic year, so that it could be reported to the Society. In the third place, a meeting in June or July would mean equable climatic conditions during the sessions, and the attending members would be less likely to experience in a few days, more or less unprepared, samples of all the seasons as at present during the Christmas holidays.

There are, of course, objections to this suggested change. The gravest one, perhaps, is the fact that most of the Societies forming the Federation have clauses in their constitutions which fix the annual session in the last week of December and the first week of January. Now, without losing time in deploring the tendency to regulate and direct every manifestation of life in a Society by constitutional pro-

¹ The Easter holidays are not suitable as a meeting period because not all colleges and universities give a vacation, nor do the vacations coincide in time.

visions, it may be remarked that even the necessity of a constitutional amendment should not permanently block an improvement. It must, however, be admitted that constitutions are not easily amended, that the channels to this fortress are tortuous and often mined, so that the unwary navigator is frequently blown up with astonishing ease by the orthodox defenders of the citadel.

This matter of altering the time of meeting has been mentioned here, not because of its novelty, for it has been discussed lightly on several occasions in the past, but in the hope that a majority of the Federation will take it under serious advisement.

Attendance. The attendance was excellent in general, but geographically it was ill-balanced, the eastern section of the country being represented by relatively few men. This absence, flatteringly enough for the Atlantic seaboard, caused a few subacid remarks.

Entertainment. TRIP AROUND ST. LOUIS. On Wednesday afternoon the Local Commit. arranged a series of enjoyable visits to the St. Louis Hospitals and laboratories, and also to the beautifully located and impressive buildings of Washington Univ.

Vote of thanks. At the last executive session of the Pharmacol. Soc. a motion was passed unanimously to thank the authorities of Washington Univ. for their hospitality and the Local Commit. for its broad and efficient efforts to render the stay of their guests in St. Louis as pleasant and profitable as possible.

V. AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY: SECOND ANNUAL MEETING

G. H. Whipple, Secretary

At the second annual meeting of the Pathol. Soc., in addition to participation in the three sessions of the Federation, papers were presented at an independent session.

Scientific program. MONDAY, DEC. 28, 2 p. m. *E. C. Rose-now*: Studies on streptococci.—*B. S. Kline* and *S. J. Meltzer*: The effect of previous intravenous injections of the pneumococcus upon experimental pneumonia by intra-bronchial insufflation of the same organism.—*Ludvig Hektoen*: Observations on the formation of anti-bodies.—*Leo Loeb*: Autoplastic and homoplastic transplantation of tissues.—*H. T. Karsner*: Further studies in nitrogen retention and renal function.—*H. G. Wells*: Metastatic calcification.—

G. H. Whipple and *C. W. Hooper*: Studies in bile pigment excretion.—*C. W. Duval*: Further studies upon the experimental production of leprosy in the lower animal.—*E. L. Opie* and *L. B. Alford*: The influence of diet upon the progress of a bacterial infection.—*G. W. Crile*, *F. W. Hitchings* and *J. B. Austin*: Pathological lesions wrought by certain amino-acids, by skatol and indol, by iodine, foreign proteins, and certain organic acids,—and the control of the action of these agents by morphia.

Executive proceedings. OFFICERS-ELECT: President—*Theobald Smith*; Vice-President—*G. H. Whipple*; Secretary-treasurer—*Peyton Rous*; Councillor—*R. M. Pearce* vice *Harvey Cushing*, term expired.

NEW MEMBERS: *James B. Murphy*, Rockefeller Inst.; *L. G. Rowntree*, Johns Hopkins Hosp.; *Richard Strong*, Harvard Med. Sch.; *M. C. Winternitz*, Johns Hopkins Med. Sch.

ADDENDUM

The following papers of biochemical interest were read at the thirty-first meeting of the Amer. Assoc. of Anatomists, which was held at the Washington Univ. Med. Sch., in St. Louis, Mo., Dec. 28–30, 1914, in conjunction with the Federation of Amer. Soc. for Exp. Biology:

C. M. Jackson: Effects of acute and chronic inanition upon the relative weights of the various organs and system of adult albino rats.—*C. M. Jackson*: Changes in young albino rats held at constant body-weight by underfeeding for various periods.—*R. M. Strong*: Further observations on the origin of melanin pigments.—*G. W. Bartelmez*: Some effects of mammalian thyroid and thymus glands upon the development of amphibian larvae.—*Preston Kyes*: Morphological evidence of intracellular destruction of red blood corpuscles.—*Montrose T. Burrows*: An attempted analysis of growth.—*R. M. Strong*: Microscopic slides showing feather germs with dermal pigment.—*Eduard Uhlenhuth*: Is function and functional stimulus a factor in producing and preserving morphological structures?—*E. I. Werber*: Is defective and monstrous development due to parental metabolic toxemia?—*J. F. Gudernatsch*: Feeding experiments on rats.

*Laboratory of Biological Chemistry of Columbia University,
College of Physicians and Surgeons, New York.*

PAPERS OF BIOCHEMICAL INTEREST, PRESENTED
BEFORE THE AMERICAN ASSOCIATION FOR
THE ADVANCEMENT OF SCIENCE, AND
AFFILIATED SOCIETIES, PHILA.,
DEC. 28, 1914—JAN. 1, 1915

SELECTED BY
JOSEPH S. HEPBURN

American Association for the Advancement of Science.
GENERAL SESSION.—*E. B. Wilson*: Some aspects of progress in modern zoology (annual address of the retiring president).

SECTION C (CHEMISTRY).—*P. A. Maignen*: Chemical preservation of manure.—*C. P. Fox*: Character of the glutinous contents of the fruit of the American mistletoe.

JOINT SESSION OF SECTIONS C AND K, AND THE SOCIETY OF AMERICAN BACTERIOLOGISTS.—*C. L. Alsberg*: Theories of fermentation (vice-presidential address, Section C).—*C. S. Hudson*: Enzyme action.—*A. I. Kendall*: Rôle of microorganisms in the intestinal canal.—*F. P. Gorham*: Use of bacteria in the treatment of textile fibres.—*C. E. Marshall*: Micro-organisms in their application to agriculture.

SECTION F (ZOOLOGY) AND AMERICAN SOCIETY OF ZOOLOGISTS.—*E. P. Churchill*: The absorption of fat by fresh-water mussels.—*G. A. Baitzell*: On a certain fibrin reaction which occurs in living cultures of frog tissues.—*E. N. Harvey*: Studies on the phosphorescent substance of the fire-fly (p. 212).

SECTION G (BOTANY) AND THE BOTANICAL SOCIETY OF AMERICA.—*J. C. Bose*: Plant autographs.

SECTION I (SOCIAL AND ECONOMIC SCIENCE).—*L. F. Bishop*: The bearing of diet on efficiency in brain workers after forty.

SECTION K (PHYSIOLOGY AND EXPERIMENTAL MEDICINE).—*Theo. Hough*: The classification of nervous reactions (vice-presidential address).

SECTION K AND SOCIETY OF AMERICAN BACTERIOLOGISTS; JOINT SESSION. Symposium on ventilation.—*A. C. Abbott*: Air-borne

diseases.—*E. B. Phelps*: Fundamental physical problems of ventilation.—*C.-E. A. Winslow*: Standards of ventilation—hygienic and esthetic.—*D. D. Kimball*: Modern developments in air conditions.

SECTION L (EDUCATION).—*Louise S. Bryant*: Blood pressure among feeble-minded people.

American Society of Naturalists. *G. G. Scott*: Some indications of the evolution of the osmotic pressure of the blood and other body fluids.

Botanical Society of America. *W. J. V. Osterhout*: The chemical dynamics of living protoplasm; The nature of mechanical stimulation; The nature of antagonism.—*G. B. Reed*: Studies on plant oxidases.—*A. R. Davis*: Enzymes of the marine algae.—*C. O. Appleman*: Concerning the measurement of diastase activity in plant extracts.—*M. C. Merrill*: Electrolytic determination of exosmosis from the roots of anesthetized plants; Some relations of plants to distilled water and certain dilute toxic solutions.—*Mr. Kno*: Influence of certain salts on nodule production in the vetch.—*J. K. Wilson*: Physiological studies of *Bacillus radiciola* of soy bean.—*Lewis Knudson*: Direct absorption and assimilation of carbohydrates by green plants.—*R. H. True* and *H. H. Bartlett*: The absorption and excretion of electrolytes by *Lupinus albus* in dilute simple solutions of nutrient salts; The absorption and excretion of electrolytes by *Lupinus albus* in dilute solutions containing mixtures of nutrient salts.—*H. S. Reed* and *H. S. Stahl*: A preliminary study of the chlorophyl compounds of the peach leaf.—*L. A. Hawkins*: Some effects of the brown-rot fungus upon the composition of the peach.

American Phytopathological Society. *Caroline Rumbold*: Some effects on chestnut trees of the injection of chemicals.

Society for Horticultural Science. *W. H. Chandler*: Some problems connected with killing by low temperatures.

Society of American Bacteriologists. *Jean Broadhurst*: Some induced changes in streptococci.—*I. J. Kligler*: A study of the correlation of the agglutination and fermentation reactions among the streptococci.—*N. S. Ferry*: The filterability of *B. bronchisepticus*, with an argument for a uniform method of filtration.—*Zae Northrup*: The influence of the concentration of the gelatin in gelatin media upon liquefying and non-liquefying bacteria.—*M. R.*

Smirnow: Induced variations in chromogenesis; induced variations in the cultural characters of *B. coli*.—*K. F. Kellerman* and *N. R. Smith*: Halophytic and lime-precipitating bacteria.—*K. F. Kellerman* and *R. C. Wright*: Relation of crop to bacterial transformation of nitrogen in the soil.—*F. W. Turner* and *L. V. Burton*: A note on the occurrence and classification of the gas formers in nature.—*Charles Thom*: The bacteriological work of the Bureau of Chemistry and its possibilities.—*R. S. Breed*: The standard method of determining nitrate reduction.—*E. B. Vedder*: A culture medium for growing gonococci and tubercle bacilli.—*S. A. Petroff*: A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces, with the aid of sodium hydrate and gentian violet-egg-meat juice media.—*R. G. Colwell*: Comparative tests of various peptones.—*F. M. Scales*: The preparation of cellulose for cellulose agar.—*P. E. Brown*: The solution versus the soil method for the bacteriological examination of soils.—*S. H. Ayers* and *Philip Rupp*: The alkali forming bacteria found in milk.—*C. W. Brown*: Degradation of casein in the presence of salt by butter flora.—*R. E. Buchanan* and *B. W. Hammer*: Bacteriology of slimy milk.—*K. Peiser*: Factors influencing the resistance of lactic acid bacteria to pasteurization.—*Maud M. Obst*: Bacteria in preserved eggs.—*C. G. Supplee*: Efficiency of Endo's medium in detecting members of the colon group.—*J. Vanderleck*: Bacteria which produce black colonies on aesculin-bile-salt agar plates and do not belong to the colon group.—*C. Greathouse*: Numbers and efficiency of *Bacillus bulgaricus* in commercial preparations from January to June, 1914.—*C. N. Hilliard*: The death rate of bacteria upon drying.—*L. F. Rettger* and *T. G. Hull*: The influence of milk and carbohydrate feeding on the bacteriology of the intestine.—*John Weinzirl*: A bacteriological method for determining manural pollution of milk.—*Thomas W. Melia*: Some observations with the use of bile media.—*A. J. Smith* and *M. T. Barrett*: Oral endamebiasis.—*Chas. Krumwiede, Jr.* and *Josephine Pratt*: Methods of isolation and differentiation of the typhoid-paratyphoid-enteriditis group.—*G. H. Smith*: The production and detection of specific ferments for the typhoid-coli group.—*J. F. Siler*, *P. E. Garrison* and *W. J. MacNeal*: Recent studies of pellagra.—*J. A. Kolmer* and *Emily Moshage*: The Schick test for diphtheria.—*J. B. Bronfenbrenner*: The

mechanism of the Abderhalden reaction (p. 87).—*D. H. Bergey*: Do bacteria produce pyrogenic poisons?—*E. C. L. Miller*: How bacterial vaccines act.—*P. B. Hadley*: Reciprocal relations of virulent and avirulent cultures in active immunization.

Philadelphia, Pa.

SCIENTIFIC MEETINGS OF THE COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION, AT THE COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK¹

PROCEEDINGS REPORTED BY THE SECRETARY,
EDGAR G. MILLER, JR.

I. EIGHTEENTH (FIFTH ANNUAL) MEETING

The *eighteenth scientific meeting* of the Columbia Univ. Biochem. Assoc. was held in the Library of the Columbia Med. Sch., at 8:15 P. M., on June 1, 1914. Abstracts of the papers are presented here (pages 194, 203) in two groups: (A) *Abstracts of papers on research by non-resident members*² and (B) *abstracts of papers from the Columbia Biochem. Dep't and affiliated laboratories*. The appended summary facilitates reference to the abstracts (133-151).³

A SUMMARY OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE SUCCEEDING ABSTRACTS (133-151).

A

- J. BRONFENBRENNER and J. ROCKMAN.
A note on the use of purified antigen of Besredka in the serum diagnosis of tuberculosis. (133)
- J. BRONFENBRENNER and J. ROCKMAN.
The diagnostic value of the Landau test for syphilis. (134)
- J. BRONFENBRENNER and J. ROCKMAN.
Further studies on Besredka tuberculin. (135)

- J. BRONFENBRENNER, W. T. MITCHELL, JR., and M. J. SCHLESINGER. Studies on so-called protective ferments. I. The sensitization of substratum for the Abderhalden test. (136)
- I. J. KLIGLER. Observations on the metabolism of *Bacillus vulgaris*. (137)
- I. J. KLIGLER and V. E. LEVINE. The Scheurlen-Klett selenium reaction in the diphtheria group. (138)

¹ Scientific meetings are held *regularly* on the first Fridays of December, February, and April, and on the first Monday in June. Proceedings of the sixteenth and seventeenth meetings were published in the last number of the *BIOCHEMICAL BULLETIN*, 1914, iii, pp. 454 and 465.

² Members of the Association who were not *officially* connected with the Columbia Biochemical Department when the researches were conducted.

³ Previous abstracts were published in the *BIOCHEMICAL BULLETIN*: 1-44, 1912, ii, p. 156; 45-62, 1913, ii, p. 285; 63-72, 1913, ii, p. 452; 73-85, 1913, ii, p. 462; 86-107, 1913, ii, p. 541; 108-119, 1914, iii, p. 302; 120-126, 1914, iii, p. 454; 127-132, 1914, iii, p. 465. See also pages 210, 224 and 228.

MAX KAHN and J. SUBKIS. On the presence of oleic and other unsaturated acids in the gastric contents. (139)

ALWYN KNAUER and BENJAMIN HOROWITZ. A volumetric determination of sulfates in urine. (140)

SERGIUS MORGULIS. The respiratory exchange of fish. (141)

ANTON R. ROSE and KATHERINE R. COLEMAN. A standard for the determination of ammonia by means of Nessler solution. (142)

ANTON R. ROSE and KATHERINE R. COLEMAN. A micro-urease method for the determination of urea. (143)

ANTON R. ROSE and ARTHUR KNUDSON. The influence upon metabolism of feeding *B. coli*. (144)

MATTHEW STEEL. A further study of

the influence of electricity on metabolism. (145)

B

O. C. BOWES. Studies in goat metabolism. (146)

RUTH S. FINCH. On the precipitation of proteins with solutions of chromates. (147)

MARK J. GOTTLIEB and SEYMOUR OPPENHEIMER. Active immunization to hay fever. (148)

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A. ABSTRACTS OF PAPERS BY NON-RESIDENT MEMBERS⁴

133. A note on the use of purified antigen of Besredka in the serum diagnosis of tuberculosis. J. BRONFENBRENNER and J. ROCKMAN. (*Pathol. and Research Lab., West. Penn. Hosp., Pittsburgh, Pa.*) Published in the preceding issue: *BIOCHEM. BULL.*, 1914, iii, p. 375.

134. The diagnostic value of the Landau test for syphilis. J. BRONFENBRENNER and J. ROCKMAN. (*Pathol. and Research Lab., West. Penn. Hosp., Pittsburgh, Pa.*) Published in the preceding issue: *BIOCHEM. BULL.*, 1914, iii, p. 377.

135. Further studies on Besredka tuberculin. J. BRONFENBRENNER and J. ROCKMAN. (*Pathol. and Research Lab., West. Penn. Hosp., Pittsburgh, Pa.*) Published in the preceding issue: *BIOCHEM. BULL.*, 1914, iii, p. 381.

136. Studies on so-called protective ferments. I. The sensitization of substratum for the Abderhalden test. J. BRONFENBRENNER, W. T. MITCHELL, JR., and M. J. SCHLESINGER. (*Pathol. and Research Lab., West. Penn. Hosp., Pittsburgh, Pa.*) Published in the preceding issue: *BIOCHEM. BULL.*, 1914, iii, p. 386.

⁴ Members of the Association who were not *officially* connected with the Columbia Biochemical Department when the researches were conducted.

137. Observations on the metabolism of *Bacillus vulgaris*.

I. J. KLIGLER. (*Dep't of Public Health, Amer. Museum of Natural Hist., N. Y. City.*) *B. vulgaris*, ever since its discovery by Hauser, has been associated with putrefaction. Hauser, Tisier and Metchnikoff attribute to it putrefactive properties. Metchnikoff believes it is the etiologic factor in infant diarrhea. Bienentock, and more recently Rettger, deny, however, that this organism has the power of initiating real putrefactive processes. Aside from these conflicting results, comparatively little is known of the metabolism of *B. vulgaris* and the various conditions influencing it.

Glenn observed that glucose exerts an inhibiting influence on liquefaction, and attributed the inhibition to the acid produced. Detailed experiments, though as yet incomplete, indicate that, while the acid does inhibit the action of the liquefying enzyme, in the carbohydrate medium it is really the sugar that is directly responsible for the absence of liquefaction. The sugar has a decided sparing effect on the nitrogenous metabolism. The liquefying enzyme is either not secreted during the early stages of the carbohydrate metabolism or else must be inactivated in some way by the acid. If the acid produced by the organism in a 1 percent sugar-gelatin sol. is neutralized, the medium shaken with toluene to kill the bacteria, and the tubes incubated, no liquefaction is obtained. This test was made with a number of strains after growing them for about three weeks on the sugar medium, which was thus rendered highly acid (5-6 percent *N* acid). Further evidence of the sparing action of the sugar is obtained from the end-products in a parallel series of gelatin with, and without, sugar. The former gave high acidity, no odor, and slight amounts of indol and ammonia; while the latter gave slight acid reaction, marked fecal odor, with large amounts of ammonia and indol. The enzyme itself is active both in weak alkaline and acid solutions (— 1 percent *N* NaOH; + 2 percent *N* HCl), but is inhibited by higher concentrations.

The oxygen relation of the organism is very interesting. It is generally supposed to be a facultative anerobe. Preliminary experiments indicate, however, that only in the presence of a utilizable sugar can it grow in the absence of oxygen. The sugar molecule apparently supplies the oxygen. In the absence of sugar, when the protein has to be utilized for nutrition, oxygen in an available

form is essential. It is noteworthy in this connection that lactose is not fermented under aerobic conditions but is broken down under anaerobic conditions.

My experiments on the putrefactive properties of this species thus far bear out Rettger's conclusions. *B. vulgaris* does not decompose coagulated meat and egg-albumen, either in the presence or absence of oxygen. *B. vulgaris* plus *B. putrificus* produce active putrefaction under aerobic conditions, the *vulgaris* apparently using up the dissolved oxygen, thus enabling the *putrificus* to act. That this is the case is indicated by the inhibition of decomposition for several days, in tubes containing added glucose (0.5 percent). With sugar present, the nitrogenous metabolism is very slight and oxygen is not removed from the solution.

The results thus far show a very delicate physiologic adjustment to the food environment by the organism studied. Seven strains of *B. vulgaris* were used and, barring certain individual variations, they behaved uniformly in the essential points.

138. The Scheurlen-Klett selenium reaction in the diphtheria group. I. J. KLIGLER and V. E. LEVINE. (*Dep't of Public Health, Amer. Museum of Natural Hist., N. Y. City.*) Reduction is a property of living cells, including bacteria. Some forms of bacteria do not reduce as readily as others; some do not reduce at all. The phenomenon of reduction is utilized in differentiating these types, nitrates being generally used for the purpose. Recently selenium and tellurium have been employed in treatment of various pathological conditions; and a number of workers, notably Scheurlen, Klett, Gosio, Glöger and Maassen, having tested the effect of selenium or tellurium compounds on bacteria, report reduction by most common forms. Glöger found, however, that diphtheria and pseudo-diphtheria organisms among others do not reduce. We have tested the effect of this group of bacteria on selenium dioxid, and sodium selenite.

Four strains of *B. diphtheria*, seven strains of *B. pseudo-diphtheria*, and three strains of diphtheroid organisms from Hodgkin's disease were used. These were grown on agar slants containing 1 part of selenium dioxid, or 1 part of sodium selenite, in 200,000, 100,000, 50,000, 25,000 and 10,000 parts of agar, respectively. Reduction was induced by all organisms, but no action was observed

in dilutions above 1 : 100,000. Dilutions of 1 : 25,000 and 1 : 10,000 gave the best results. Reduction occurred only on the surface and the growth was colored brick red, due to the deposition of selenium particles, which, under the microscope, appeared to be in the cell, indicating that the reduction was intracellular. After a few days the color began to fade and a characteristic odor of volatile selenium was produced. None of the organisms was inhibited in growth by any of the dilutions used. There were no sharp distinctions in types of growth of the various strains, though the diphtheria bacilli gave characteristic discreet colonies, which differed markedly from those of the pseudo-diphtheria. This was especially evident in the higher concentrations after 12 to 18 hr. growth. It is uncertain whether the difference is sufficiently constant to be of diagnostic value. The experiments are in progress.

139. On the presence of oleic and other unsaturated acids in the gastric contents. MAX KAHN and J. SUBKIS. (*Chem. Lab., Beth Israel Hosp., N. Y. City.*) After administering to the patient a piece of bread and a glass of water, and withdrawing the gastric contents, the authors determined, by Hübl's method, the amount of iodine absorbed by the filtrate. Gastric contents of low acidity had a small iodine number; of high acidity, a relatively large iodine number. These results contradict Graf, who stated that the gastric juice of patients suffering from carcinoma of the stomach contains an appreciable amount of oleic acid.

140. A volumetric determination of sulfates in urine. ALWYN KNAUER and BENJAMIN HOROWITZ. (*Physiol. Lab., Fordham Univ. Med. Sch., N. Y. City.*) Solutions required: (1) Barium chlorid, 1 cc. = 0.005 gm. of sulfuric acid; (2) potassium sulfate, 1 cc. = 1 cc. of sol. (1).

I. *Total (inorganic and ethereal) sulfates:* 50 cc. of urine and 5 cc. of hydrochloric acid sol. (sp. gr., 1.2) are poured into an Erlenmeyer flask, and the mixture boiled for about 5 min. Barium chlorid sol., 1, usually 10–15 cc., is then added and the mixture boiled 3–4 min. longer. Three small test tubes, of uniform bore and perfectly clean, are now placed side by side in a test tube rack, and labelled *A*, *B* and *C*. Small portions (not more than 1 cc.) of the sol. are filtered into each, using a very small funnel and a very small filter paper for the purpose. *A* is kept as a control. To *B* 3 drops

of barium chlorid sol., and to *C* 3 drops of potassium sulfate sol., are added. If not enough barium chlorid sol. has been added, *B* will be cloudy; if barium chlorid is in excess, then cloudiness appears in *C*. In either case the contents of the test tubes are carefully poured back into the main sol., the test tubes and filter being thoroughly rinsed with dist. water, and 1 cc. of barium chlorid or potassium sulfate sol., depending upon which is in excess, is now added. The tests are repeated. If, in the first trial, test-tube *B* gave a cloudiness and, after the addition of another cc. of barium chlorid sol., it still continues to give a cloudiness (whereas *C* is clear or far less cloudy than *B*), there is evidently an excess of SO_4 ions, and therefore more barium chlorid is added. If the further addition of barium chlorid causes the reverse to take place, namely, clearness or slight cloudiness in *B* and decided cloudiness in *C*, there is an excess of Cl ions, and therefore more potassium sulfate is added. It is evident that if, with a given vol., the results are the reverse of those seen with 1 cc. less, the end point must lie somewhere between these two volumes. If the determination need be approximate only, then the average of the volumes is taken. If not, further trials, with successive 0.1 cc., are made, until a point is reached where 3 drops each of barium chlorid and potassium sulfate sol. added to *B* and *C* give precisely the same cloudiness. To confirm this endpoint, take a fresh sample of urine, and add to it at once the total volume of barium chlorid sol. Samples of the filtrate should give equal cloudiness with barium chlorid and potassium sulfate.

II. *Inorganic sulfates*. The procedure is analogous to the above, except that acetic is substituted for hydrochloric acid, and the solution is *not heated*.

I — II = Ethereal sulfates.

III. *Neutral sulfur*. Here 50 cc. of urine are treated with 5 gm. of potassium nitrate and 7 gm. of sodium carbonate. The mixture is evaporated and then heated till the carbonaceous mass is completely oxidized. The residue is dissolved in water, the sol. poured into an Erlenmeyer flask, neutralized with hydrochloric acid and 5 cc. of hydrochloric acid sol. (sp. gr., 1.2) added. From here follow I. This will give total sulfur. If the total-sulfate sulfur (obtained in I) is subtracted from this, the result will be neutral sulfur.

141. The respiratory exchange of fish. SERGIUS MORGULIS.

(*U. S. Fisheries Biological Station, Woods Hole, Mass.*) Apart from the technical difficulties involved in the investigation of the gaseous metabolism of aquatic animals, the inability to control their behavior is the strongest drawback in such studies. It is a well known fact that muscular exertion of any kind increases the gaseous exchange very considerably; and unless a uniform base line, unaffected by bodily movements, can be established, the influence of different factors cannot be determined with any degree of accuracy.

To overcome this latter difficulty I have chosen, for experiment, the flounder, which normally does not move about but rests, sometimes for hours, in the same position. The flounder, and a few other fish, have the habit of lying quietly on the bottom of the receptacle without changing their positions and, if not disturbed, move neither fins nor tail. These aquatic animals are therefore practically ideal subjects for metabolism investigations.

Owing to lack of special apparatus for determining the carbon-dioxid output and oxygen consumption, I was obliged to limit my research to the oxygen alone. Determination of the carbon dioxid in sea-water, by an easily available method, is still an unsolved problem. Oxygen, on the contrary, can be measured very accurately by means of the Winkler method. The latter is described in detail in special manuals and need not be given here. It will suffice to say that it is an iodometric method and requires very little time. As an illustration of the accuracy of the method I may quote a few duplicate analyses of the oxygen content of sea-water in the Laboratory of the Bureau of Fisheries at Woods Hole. On different days the following results were obtained, expressed as cc. of oxygen per liter:

<i>A</i>	<i>B</i>	<i>C</i>
5.58	5.95	5.52
5.54	5.95	5.55

The method of studying the oxygen consumption by the flounder was very simple. The fish, usually of small size, was put in a vessel of known volume filled with sea-water, of which a sample was analyzed for oxygen. The vessel was then tightly closed, the time recorded, and temperature of the water noted. From the percentage of oxygen the total quantity dissolved in the vessel

could be ascertained. After a period varying from one to several hours, the vessel was opened and a sample of the water again analyzed. The residual oxygen in the vessel could thus be calculated; and the difference between the first and second amounts gave the quantity of oxygen consumed by the flounder during the experiment.

By this method it was found that the relative rate of oxygen consumption increased as the size of the flounder diminished. Thus, it was found that, with the body weights varying as 18:6:1, the oxygen consumption was in the ratio of 1:1.3:1.33. It was found, also, that the consumption of food invariably caused an increase in the oxygen intake by 25 to 30 percent.

In the case of one small flounder, which weighed 3.75 gm., it was found that the oxygen consumption per hour showed great regularity and a tendency to decrease in the course of a seven-day fast, as may be seen from the following data:

Date	Weight, grams	Oxygen per hour, cc.
IX 5	3.75	0.492
6	—	0.359
7	—	0.368
8	—	0.398
9	—	0.271
10	—	0.207
11	—	0.226
12	3.43	0.230

We observe a very abrupt drop in the oxygen consumption per hour, on the first day of fasting, which then remains fairly constant for the next three days. On the fourth and fifth days again a rather rapid decrease is seen, the oxygen intake per hour being now less than one half of that found 24 hr. after the last previous feeding.

The entire experiment lasted 171.5 hr., of which the flounder spent fully one third in the respiration vessel. In that time it used up a total of 56.5 c.c. of oxygen. The loss in body weight for the same length of time was 0.32 gm. or 8.5 percent. It is noteworthy that the amount of substance which could be oxidized by 56.5 c.c. of oxygen is considerably *less* than the loss in body weight observed. The fact is significant, especially if we recall that Pütter⁵ has main-

⁵ Pütter: *Zeitschr. f. allg. Physiol.*, 1909, ix, p. 147.

tained that the loss in weight is usually insufficient to account for the amount of oxidation and has postulated, therefore, the theory of a nutritive value for aquatic animals of substances dissolved in the water.⁶

142. A standard for the determination of ammonia by means of Nessler solution. ANTON R. ROSE AND KATHERINE R. COLEMAN. (*Research Laboratory, Fenton B. Turck, M.D., Director, N. Y. City.*) Published in the preceding issue: *BIOCHEM. BULL.*, 1914, iii, p. 407.

143. A micro-urease method for the determination of urea. ANTON R. ROSE and KATHERINE R. COLEMAN. (*Research Laboratory, Fenton B. Turck, M.D., Director, N. Y. City.*) Published in the preceding issue: *BIOCHEM. BULL.*, 1914, iii, p. 411.

144. The influence upon metabolism of feeding *B. coli*. ANTON R. ROSE and ARTHUR KNUDSON. (*Research Laboratory, Fenton B. Turck, M.D., Director, N. Y. City.*) Bouillon inoculated with *B. coli* was fed to dogs in a basal ration of meat, cracker meal and lard. There was a somewhat pronounced change in the composition of the urine during the first week, but later a gradual tendency towards the status of the normal periods. The amounts of sulfur and nitrogen ran parallel. The elimination of these elements in the urine was temporarily decreased. There was marked diminution of neutral sulfur with an increase of sulfate sulfur. The etheral sulfur rose immediately and then gradually subsided to the same plane as in the preliminary period. Feeding of *B. coli* was followed by pronounced increase in indican in the urine, but this soon disappeared, and protracted feeding of the bacteria did not bring it back. In general, the introduction of *B. coli* caused disturbance, but there was readjustment in the course of two or three weeks.

145. A further study of the influence of electricity on metabolism.⁷ MATTHEW STEEL. (*Long Island College Hospital.*) The present research consists of five experiments, of 9 to 12 days each. Four different kinds of electrical modalities were used. The subject was a normal healthy adult, and the diet was non-purin and uniform for each experiment.

⁶ Further results of this research have lately been published in detail in the *Journal of Biological Chemistry*, 1915, xx, p. 37.—(ED.)

⁷ Steel: *BIOCHEM. BULL.*, 1914, iii, p. 309.

Experiments I and II. Autocondensation current, with thick dielectric. Treatment: 500 m. amp. for 30 min. The following symptoms were noted: Fall in blood-pressure, 4 to 10 mm.; slight rise in pulse-rate; increase in the daily vol. of urine, 100 to 300 c.c.; and increase of 5 to 6 gm. of total urinary solids per day. There were slight increases in the quantities of all the nitrogen constituents, the greatest increases being in urea and creatinin nitrogens.

Experiment III. Combination of direct d'arsonval current and the autocondensation current, with thin dielectric. Treatment through the feet, 1500 m. amp., 5 min.; through the hands and feet, 1750 m. amp., 15 min.; through the hands and feet, 1960 m. amp., 6 min.; through the hands and feet, 1600 m. amp., 4 min. The following symptoms were noted: Fall in pulse-rate and blood-pressure; gentle warmth beginning at the wrists and gradually extending over the entire body; slight flushing of the capillaries, especially of the skin of the hands and wrists; rise in body temperature of 1° F.; decrease in the daily vol. of urine, 200 to 300 c.c.; and increase in total urinary solids of about 2 gm. per day; slight increase in the quantities of all the nitrogenous constituents.

Experiment IV. Static wave current. Treatment: a large metal plate was placed over the liver for 15 min., then over the kidneys for 15 min. The following symptoms were noted: There was a small increase in the daily vol. of urine; increase in total urinary solids of 6 to 7 gm. per day; and increase of 0.82 gm. of total urinary nitrogen per day. The urea nitrogen was increased 0.76 gm.; the other nitrogen constituents were increased slightly.

Experiment V. Galvanic sinusoidal current. Treatment: 70 m. amp., through the back and abdomen, for 30 min. This modality caused decrease in the daily vol. of urine, 400 to 500 c.c.; increase of about 1 gm. in total urinary solids per day; and small increase in the amounts of all the nitrogen constituents.

In each of the above experiments the urine voided during the fore periods did not respond to the urorosein nor the nitrite tests, whereas the urine voided during the periods of electrical treatments responded strongly to each test. The urine voided during the after periods responded slightly.⁸

⁸ Steel: *Loc. cit.*

B. ABSTRACTS OF PAPERS FROM THE COLUMBIA BIOCHEM. DEPT

146. Studies in goat metabolism. O. C. BOWES. The following data relate to the preliminaries in a study of goat metabolism. The animal was placed in a cage of the kind in regular use in this laboratory in experiments on dogs. For the collection of the excreta certain modifications of the cage were made. The meshes of the wire platform, for example, on which the animal stood, were $7/8$ in. square. A special deep drip pan conveyed the excreta to a short chute with a screen of wire netting in the bottom, through which the urine passed into a container, the feces rolling to the end of the chute into a separate receiver and thus affording a very satisfactory separation of the latter from the former.

A separate analysis was made of each feed in the ration, which included weighed quantities of hay, oats, bran, corn meal and linseed meal. The coefficient of digestibility, as recorded below, was for the entire ration. A small quantity of bone ash in the diet was found advantageous for hardening the feces and thus facilitating their collection. Aliquot portions of the daily feces were promptly dried and subjected to analysis in composite samples for the whole period.

The results for 12 days of feeding the above mentioned rations, which followed a preliminary period of two weeks on the same diet, are summarized in the following table:

	Intake, grams	Output in feces, grams	Coefficient of digestibility, per cent
Total nitrogen.....	199.03	77.42	61.1
Total lipins.....	261.65	94.67	63.8
Total carbohydrates.....	9,006.68	3,698.20	59.9
Total ash.....	459.35	353.92	22.9

147. On the precipitation of proteins with solutions of chromates. RUTH S. FINCH. Following the suggestions of Dr. Gies, I have continued some unpublished work by him and Dr. Wm. H. Welker on the precipitation of proteins from acid solutions by chromates. In these preliminary experiments I have endeavored to determine the completeness of precipitation, the scope of application, and the possible practical uses, of this method.

When 5 cc. of fresh watery extract of liver are treated with 5

drops each of 10 percent acetic acid and 10 percent potassium chromate sol., a heavy flocculent, yellow-brown precipitate is produced, which is easily separated by filtration.⁹ Excess of chromate may be removed from the clear filtrate with a few drops of 10 percent barium chlorid sol., after nearly neutralizing the acid with ammonium hydroxid. When filtered through double quantitative filter paper, the clear filtrate gives no response to the biuret test, thus showing that precipitation of protein has been complete. Lithium, calcium, strontium and ferric chromates, and potassium bichromate, produce similar precipitates. Dilute mineral acids may be used instead of acetic, though boric acid is altogether too weak. Too much acid prevents clear filtration and too much chromate seems to dissolve the precipitate.

Fresh extracts of most of the tissues of the body, as kidney, brain, lung, intestine, stomach, and heart give similar precipitates. Fresh blood must be diluted 1-5 or 1-10, and treated with the proportions of 15 cc. of acid and 10 cc. of chromate sol. per 100 cc. of diluted fluid, to give complete precipitation. Milk and dilute egg-white give very heavy precipitates but the filtrates are not clear unless the phosphates are first eliminated. Since the filtrate from egg-white always contained some protein, ovomucoid was prepared and purified in the usual way, and its precipitability tested. It was not precipitated by the acid-chromate combination.

Of derived proteins, metaproteins obtained by acid extraction of meat were always completely precipitated. Amino acids, peptones and secondary proteoses give no precipitates. Primary proteoses, purified according to Kühne, yielded heavy yellow precipitates, but the filtrates responded weakly to the biuret test. Possibly secondary proteoses were present despite the thoroughness of purification.

Of related nitrogenous products, Adams' beef extract gives no precipitate, nor does an alcoholic extract of the total solids of urine. Of alkaloids, atropin yields an emulsion: narcein and brucin, characteristic crystals; narcotin, morphin, and apomorphin, dark flocculent precipitates.

⁹ Gies: *American Journal of Physiology*, 1903, viii; *Proceedings of the American Physiological Society*, p. xv.

Chromates may evidently be used in many relations as a qualitative protein reagent instead of potassium ferrocyanid, and they prove more advantageous under certain conditions because excess is easily eliminated from solution. They should be useful as *quantitative* precipitants of various proteins. We have used the method successfully to remove protein from solutions prior to the isolation of such associated substances as ovomucoid and glycogen. It is our intention to continue this study.

148. Active immunization to hay fever. MARK J. GOTTLIEB and SEYMOUR OPPENHEIMER. Published in this issue: *BIOCHEM. BULL.*, 1915, iv, p 127.

149. Nitrogen metabolism in experimental uranium nephritis. HERMAN O. MOSENTHAL. Nitrogen metabolism in experimental uranium nephritis bears certain resemblances to that in clinical nephritis. Urinary nitrogen may, in both of these conditions, be increased or diminished from the outset of the kidney involvement. It appears certain that retention of nitrogen is due to insufficient excretory powers of the kidney. Increased excretion is, in the experimental type of the disease at least, not due to previous nitrogen retention but to increased protein catabolism. This is proved by the fact that in dogs poisoned with uranium, urinary nitrogen is present in excess as compared with the intake, while at the same time non-protein nitrogen of the blood is markedly increased in amount.

A study of other phases of nitrogenous metabolism, when the blood and urine present the phenomena just stated, shows that the fecal nitrogen is unchanged in amount, and the quantity of nitrogen in the succus entericus, as determined by the Thiery-fistula method, tends to diminish. During the nephritic period, blood-pressure rises and remains above normal after all signs of renal disease disappear. Other urinary constituents—chlorids, sulfur and phosphates—fail to parallel the nitrogen in excretion. Even individual urinary nitrogenous fractions, *e. g.*, uric acid, may be retained while total nitrogen is increased.

These facts indicate that uranium nephritis implicates the body as a whole and not the kidneys only. Furthermore, the various functions of the body do not necessarily supplement each other by

vicarious excretion, etc., as is often assumed, but each one to a great extent follows its own independent laws. Human nephritis presents such a many-sided picture that probably many of the facts pertaining to experimental uranium nephritis are applicable to it. However, these facts should be considered as nothing more than suggestions upon which to base further inquiry.¹⁰

150. An alleged improvement of the ferric chlorid method for the determination of sulfocyanate. W. A. PERLZWEIG and WILLIAM J. GIES. Several years ago Bunting proposed the following method for the quantitative determination of sulfocyanate in saliva:¹¹ "Pour 5 cc. of saliva into a thin curved watch-crystal about 3 in. in diam. Allow this to stand in the air or sunlight, or better still, set it on a slowly steaming water-bath, until the saliva has dried to the dish. To this add 1 or 2 drops of water and 1 or 2 drops of ferric chlorid sol. and stir with the residue to make a thick paste. To this add 5 cc. of ether, and stir the paste thoroughly. When well mixed, hold the glass on a level with the eye and note the color of the sol." Compare, under similar conditions, with standard colors representing known conc. of sulfocyanate. Some of the serious deficiencies of this method have been shown by Dr. Kahn and the senior author.¹²

Bunting lately sought to eliminate the defects in the foregoing method by substituting for it the following procedure:¹³ "Evaporate 5 cc. of the sample (of saliva) to be tested, on a watch-crystal or small evaporating dish. To the dried film add ferric chlorid sol., drop by drop, spreading it gently with a glass rod; use just enough to moisten the whole film. Allow the mixture to stand for from 1 to 2 min., and then pour on 5 cc. of a mixture of *amyl alcohol* (5 parts) and *ether* (2 parts). Stir gently with a glass rod until all the color has been taken up by the ethereal sol. Decant the sol. into a test tube and compare with standard colors representing known conc. of sulfocyanate."

The substitution of ether-amyl alcohol mixture, for ether, was

¹⁰ The results of this research have been published in detail in the *Archives of Internal Medicine*, 1914, xiv, p. 844.—(ED.)

¹¹ Bunting: *Dental Cosmos*, 1910, lii, p. 1346.

¹² Gies and Kahn: *Ibid.*, 1913, lv, p. 40.

¹³ Bunting: *Ibid.*, 1914, lvi, p. 845.

intended to overcome the defects of the original method due to the use of ether alone, but Bunting merely evaded important deficiencies of one kind to introduce serious imperfections of another. Bunting has not proved, by any experimental procedure, that his new method is more delicate quantitatively than the conventional ferric chlorid process; he has merely indicated that the colorations obtained with his new method are more striking in some proportions—"more vivid"—than those observed with the ferric chlorid test as commonly applied. He has not shown that any given proportion of sulfocyanate which could not be detected by the conventional process would be revealed by his new method, which is the heart of the matter. He seems to have deluded himself into thinking that, because the colorations obtained with his new process are, in certain selected proportions, more intense than those for the same selected proportions with the conventional ferric chlorid test, the new method itself is more distinct, therefore more accurate, and consequently more useful. If he had followed these comparative colorations step by step to their vanishing points for each test, with adequate controls, he would have avoided this fallacy in his claims.

With the aid of the conventional ferric chlorid process, it is not at all difficult to detect 1 part of sulfocyanate—potassium salt, Kahlbaum preparation—in 4,000,000 parts of water. Neither of us has been able to do so with Bunting's process. Bunting himself claims that "by careful technic a distinct color (the yellow of ferric chlorid?) may be obtained from a sol. which contains 0.0001 per cent.—only 1 part in 1,000,000—of KCNS." *Our comparative tests with saliva have given us equally striking differences in favor of the conventional method.* All our tests were suitably controlled, of course, and very slight though significant differences in color were easily observed as a consequence.

[The senior author opened the discussion of Dr. Bunting's paper, on this "improved" method, after its original presentation at a meeting of the Academy of Stomatology of Philadelphia, March 28, 1914. The foregoing facts were presented in that discussion. For further details see *Dental Cosmos*, 1914, lvi, p. 856.

In the *printed* version of his reply to the senior author's remarks, Bunting said that "no dilution of ferric chlorid is anything but yellow" (p. 866). This

"break" shows how little Bunting knew about the disturbing influence on his test of ferric chlorid itself. He evidently failed to compare his results with those of control tests.

Bunting was asked by Gies (p. 858): "Does diacetic acid *affect* the new procedure; if so, is the disturbing effect more or less than that on the first process—possibly any contained diacetate would be decomposed by the preliminary desiccation?" To these questions Bunting replied irrelevantly as follows (p. 866): "He (Gies) then asks how the alcohol-ether method *eliminates(!)* the aceto-acetic acid, if present. Is it possible that he (Gies) does not know that the ferric salt of aceto-acetic acid is not soluble in ether?" Bunting's affected surprise in this regard would be less amusing if he had frankly answered the questions; and his chemical ignorance would be less apparent if he had addressed himself to the possible influence, on his test, of diacetic acid (in saliva) through the capacity of diacetic acid to affect the *concentration* of "soluble iron" in the final medium; to which, of course, Gies' question directly referred.

Bunting's inability, or unwillingness, to state correctly the simplest facts of the case were shown by his misuse of various remarks in his conclusion to the "discussion." Thus, Dr. Percy R. Howe, who participated in the discussion, said (p. 863): "I have tried the test many times and in my hands the color is much deeper by Bunting's ether method (the first, rejected by Bunting) than by the FeCl_3 plus H_2O ." There is no indication in this remark, or in any other that Howe made, of any determination by him of the coloric influence of excess of ferric chlorid itself in the alcohol-ether test—no suggestion of any comparison of the two tests at dilutions of sulfocyanate that might have been expected to develop the comparative values of the tests, yet Bunting says complacently and incorrectly of this remark (p. 867): "Dr. Howe told us that he had tried the method and had found it *trustworthy(!)*" On page 867 Bunting states, in another connection: "Dr. Gies has given no evidence of having made an actual test of the validity of this statement, but has contented himself with saying that it is not true." Yet the "evidence" was orally stated and appears on p. 857—printer's proof of which was submitted to Bunting prior to the publication of his own statement. (If the compliment has been returned by him, Bunting's oversight in this connection would have been pointed out before it was too late for correction.) Again, Bunting presumed to state, in his *printed* rejoinder, that Gies "says that dentists should not attempt problems which involve chemistry" (p. 867), and then worked up a ludicrous frenzy about "such a sentiment." Gies suggested, on the contrary, that "it is time for you (dentists) to eye with suspicion the expert dentist who persists in taking your time, and using space in your journals, to discuss chemical research of *doubtful* validity and of *dubious* comprehension. Let us stick to our lasts" (p. 861). There was no suggestion that dentists *qualified* to conduct chemical research should not do so.

During the discussion, Gies said: "I publicly stated, recently, to some of your colleagues at a dental meeting in New York that I have taken the war-path against your pseudo-chemists, and was told, in reply, that I might never perform a better service for dentistry." In his *printed* rejoinder to this, Bunting suggested that, should Gies continue in this direction, "he (Gies) will kill himself by his own misdirected efforts" (p. 868). Bunting is right—the more the senior author prepares himself for the execution of this purpose, especially

by reading Bunting's chemical comedies, the more he is likely to kill himself—kill himself *laughing!*

Bunting's suggestion that Gies' discussion of his (Bunting's) paper "reveals plainly the fact that he (Gies) has not done the work which he claims to have performed upon this method" leads us to propose that Gies be promptly investigated. It is suggested that the junior author be called as the first witness and that Dr. A. P. Lothrop, who tested the validity of some of our conclusions, be called as the second.]

151. Absorption of unaltered protein through the gastro-enteric tract in infants. O. M. SCHLOSS. The infants tested were given the whites of one to two eggs. The urine was collected for 6 hr. and used for precipitin tests. Urine, or protein precipitated from urine by saturation with ammonium sulfate, was injected into the peritoneal cavity of guinea-pigs and, 21 days later, similar injection of egg-white was made. The precipitin reaction was positive in very few *normal* infants, but was positive in a large percentage of those suffering from gastro-enteric disorders or malnutrition. In many instances the positive precipitin reaction was coincident with albuminuria (heat and acetic acid test) but, in a number of urines giving positive precipitin tests, no albumin was demonstrable. The anaphylactic reaction was positive in practically all cases with both albuminuria and a positive precipitin reaction, but was uniformly negative when no albuminuria was present. The results indicate that unaltered, or but slightly altered, protein can be absorbed from the gastro-enteric tract of infants suffering from nutritional disorders.

Tests were also made for protease in the blood-serum of infants. The dialytic technic of Abderhalden was used, and proteins from egg and milk were employed. Proteases were present in a few normal infants and in a large percentage of those suffering from nutritional disorders.

One of the characteristic anaphylactic reactions in the guinea-pig is a marked rise in the eosinophile blood-cells. It seemed of interest to determine whether, in the continued feeding of foreign protein, sufficient would be absorbed to cause such a reaction. Guinea-pigs were fed approximately 5 gm. of powdered egg-white a day. Four of the six animals thus fed showed marked increases in eosinophile blood-cells accompanied by leukocytosis. Control animals showed no such blood changes.

II. NINETEENTH MEETING

The *nineteenth scientific meeting* of the Assoc. was held in the Biochemical Seminar Room, at the Columbia Med. Sch., at 4:15 P. M., on Dec. 4, 1914. The appended summary facilitates reference to the abstracts (152-170) of the papers presented, pages 211, 224.

A SUMMARY OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE SUCCEEDING ABSTRACTS (152-170)

A

- J. BRONFENBRENNER. The nature of the Abderhalden reaction. (152)
- J. BRONFENBRENNER, W. J. MITCHELL, JR., and PAUL TITUS. The role of serum anti-trypsin in the Abderhalden test. (153)
- J. BRONFENBRENNER, J. ROCKMAN and W. J. MITCHELL, JR. Comparisons of urinary and serum findings in the diagnosis of tuberculosis. (154)
- J. ALEXANDER CLARKE, JR., and MARTIN E. REHFUSS. (Communicated by P. B. Hawk.) The protein content of the gastric juice in normal and pathological states. (155)
- A. F. BLAKESLEE and ROSS A. GORTNER. Reaction of rabbits to intravenous injections of mould spores. (156)
- E. NEWTON HARVEY. Studies on the photogen of luminous bacteria. (157)
- ALFRED F. HESS and MAX KAHN. Metabolism studies of two cases of hemophilia. (158)
- MAX KAHN and JACOB HOFFMANN. Calcium metabolism in normal and diabetic individuals. (159)
- MAX KAHN and ISIDORE JACOBOWITZ. A modification of the Wulf-Junghans method for the diagnosis of gastric cancer. (160)
- MAX KAHN and WILLIAM SPIELBERG. Condition of nutrition in nephrectomized patients. (161)
- I. J. KLIGLER. A study of the correla-

tion of agglutinative and fermentative characters among the streptococci. (162)

V. E. LEVINE. The reducing power of anerobes. (163)

SERGIUS MORGULIS. Body surface and metabolism of flounders. (164)

B

FREDERIC G. GOODRIDGE and MAX KAHN. The neutral-sulfur and colloidal-nitrogen tests in the diagnosis of cancer. (165)

V. E. LEVINE. Sodium selenite as a laboratory reagent for reducing substance. (166)

ALFRED P. LOTHROP and WILLIAM J. GIES, with the collaboration of HENRY W. GILLET, CHARLES C. LINTON, ARTHUR H. MERRITT and HERBERT L. WHEELER. A further study of the effects of acid media on natural extracted teeth. (167)

F. D. ZEMAN and PAUL E. HOWE. The excretion of creatin during a fast. (168)

F. D. ZEMAN and PAUL E. HOWE. Recuperation: Nitrogen metabolism of a man when ingesting successively non-protein and normal diets after a seven-day fast. (169)

F. D. ZEMAN, JEROME KOHN and PAUL E. HOWE. Variations in factors associated with acidity of human urine, during a seven-day fast and during subsequent non-protein and normal feeding periods. (170)

A. ABSTRACTS OF PAPERS BY NON-RESIDENT MEMBERS

152. The nature of the **Abderhalden reaction**. J. BRONFENBRENNER. (*Pathol. and Research Lab., West Penn. Hosp., Pittsburgh, Pa.*) Published in this issue: *BIOCHEM. BULL.*, 1915, iv, p. 87.

153. The rôle of serum anti-trypsin in the **Abderhalden test**. J. BRONFENBRENNER, W. J. MITCHELL, JR., AND PAUL TITUS. (*Pathol. and Research Lab., West Penn. Hosp., Pittsburgh, Pa.*) Published in this issue: *BIOCHEM. BULL.*, 1915, iv, p. 86.

154. Comparisons of urinary and serum findings in the diagnosis of tuberculosis. J. BRONFENBRENNER, J. ROCKMAN AND W. J. MITCHELL, JR. (*Pathol. and Research Lab., West Penn. Hosp., Pittsburgh, Pa.*) Published in this issue: *BIOCHEM. BULL.*, 1915, iv, p. 80.

155. The protein content of the gastric juice in normal and pathological states. J. ALEXANDER CLARKE, JR., AND MARTIN E. REHFUSS. Communicated by P. B. HAWK. (*Lab. of Physiol. Chem., Jefferson Med. Coll., Phila.*) The protein content of the gastric juice was investigated by the method of Wolff, namely by successively diluting the gastric juice and adding phosphotungstic reagent. It was found that the normal gastric juice, *per se*, contained only traces of protein, never giving a reaction in dilutions greater than 1:40. The protein content of the specimens removed by the fractional method, after the administration of Ewald meals, was also determined. The macerated Ewald meal *in vitro* never gave a reaction in a dilution greater than 1:40. Treated in the incubator with artificial gastric juice, the authors were able to demonstrate an increasing content due to the effect of the gastric juice on the proteins of the bread. If therefore material removed from the stomach at intervals develops a greater protein content than the theoretical content due to the action of the gastric juice on the bread proteins, we are able to say that it comes from other sources than bread. In functional states and in normal cases the protein curve followed approximately the curve for the action of the gastric juice on the Ewald meal *in vitro*. In pathological conditions, such as ulcer and cancer, the curve was entirely different. The authors pointed out the importance of differentiating the presence of blood,

infected sputum rich in proteins, and the possibility of protein rests due to deficient motility. In ulcer, a marked initial rise in the protein content was noted, which was out of proportion to the amount of acid secreted. This requires further study. In cancer the authors were able to confirm the increased protein content of cancerous achylia and to show in practically all cases *marked increase in protein concentration out of all proportion to the amount of acid present*. In cancer, this rises steadily and is usually most marked in the more advanced specimens. This *disassociation between the acidity and protein curves, the authors consider most important; it emphasized the steady rise in the protein content as digestion progresses*. The high protein content cannot be due to the action of the secreted juice on the bread but must be a special elaboration from cancerous tissue. The value of this reaction, namely the disassociation in the protein and acidity curves, is of value in direct proportion as the acidity is low and as the protein continues to diverge. In inflammatory conditions, as contrasted with functional states, the authors find a greater protein content in the former and insist on the necessity of examining the entire digestive cycle owing to the possibility of undue protein concentration at certain periods.

156. Reaction of rabbits to intravenous injections of mould spores. A. F. BLAKESLEE AND ROSS A. GORTNER. (*Storrs Agric. Exp. Station, Storrs, Conn., and Carnegie Station for Exp. Ev., Cold Spring Harbor, N. Y.*) Published in this issue: *BIOCHEM. BULL.*, 1915, iv, p. 45.

157. Studies on the photogen of luminous bacteria. E. NEWTON HARVEY. (*Physiol. Lab., Princeton Univ.*) Masses of luminous bacteria were dried on glass wool in a vacuum over calcium chlorid and ground to a powder. The powder will phosphoresce if moistened with tap-water or sea-water. Since new colonies of bacteria can usually be obtained from the powder, if planted on a suitable culture medium, *all* the bacteria are evidently not killed by drying, but *most* of them are.

If the dry powder is extracted with boiling ether for 10 hr., it phosphoresces as strongly as before, after the ether is removed and the powder moistened. Ether-treated material may give occasional

new growths on agar-agar. We may therefore conclude that the living cell is not essential for light production; and, further, that the photogen is not a fat, or a lecithin, or any ether-soluble substance.

Luminous bacteria require free oxygen in order to phosphoresce. If the bacteria could be broken up in an oxygen-free medium, any photogenic substance should dissolve in the medium and phosphoresce when oxygen is again added. The bacteria were broken up (cytolyzed) by adding (a) toluene to a bacterial emulsion in oxygen-free sea-water and (b) oxygen-free distilled water to a dense mass of bacteria in a space devoid of oxygen. After 10-15 min., oxygen was admitted but in neither case did phosphorescence appear. We may therefore conclude that the photogen is unstable and breaks up without the production of light in water free from oxygen. Of course the photogen would rapidly burn up if any free oxygen were present.

Exactly similar results were obtained with the dry powdered luminous organs of the fire-fly. (See *Jour. Amer. Chem. Soc.*, 1915, xxxvii, p. 396.)

158. Metabolism studies of two cases of hemophilia. ALFRED F. HESS and MAX KAHN. (*Chem. Lab., Beth Israel Hosp., N. Y. City.*) The intake and output of nitrogen, sulfur, phosphorus, chlorine, calcium, magnesium and fat were studied in two cases of hemophilia. It was found that in one of these cases (B. A.) there was a minus calcium balance which could be changed to a plus balance by administering calcium chlorid *per os* daily. It was found that this case also had a diminished calcium content in the blood. The other case (J.) was normal, so far as was shown by the metabolism experiments or calcium content of the blood. In the first case, we used a sol. of calcium chlorid, in saline, of such strength that the addition of 1 drop of it to 10 drops of blood exactly supplied the amount of calcium that the blood seemed to lack. It was found that the coagulation time of blood so treated was reduced from about 45 to 50 min., to 10 or 12 min.

159. Calcium metabolism in normal and diabetic individuals. MAX KAHN and JACOB HOFFMANN. (*Chem. Lab., Beth Israel Hosp., N. Y. City.*) Diabetic patients who excreted sugar in the urine showed a distinct daily calcium loss. Administration

of calcium chlorid caused a positive calcium balance. When the sugar excretion stopped, the calcium loss was much reduced. Calcium was determined by the McCrudden method.

160. **A modification of the Wulf-Junghans method for the diagnosis of gastric cancer.** MAX KAHN and ISIDORE JACOBOWITZ. (*Chem. Lab., Beth Israel Hosp., N. Y. City.*) The patient is given an Ewald test breakfast. The stomach is then thoroughly flushed, and the washings examined for nitrogen by the Kjeldahl method and for albumin by the Pfeiffer method. If the nitrogen is more than 18 mg. per 100 cc. of gastric contents and, if the albumin is more than 0.5 part per thousand, malignancy is suggested.

161. **Condition of nutrition in nephrectomized patients.** MAX KAHN and WILLIAM SPIELBERG. (*Chem. Lab., Beth Israel Hosp., N. Y. City.*) Two cases of nephrectomy were studied. The various ordinary urinary constituents were normal in proportion, except that, in one case, neutral sulfur was much increased.

162. **A study of the correlation of agglutinative and fermentative characters among the streptococci.** I. J. KLIGLER. (*Dep't of Public Health, Amer. Museum of Natural Hist., N. Y. City.*) Bacteria have evolved so little along gross structural lines that it is impossible to differentiate members of the same genus on a merely physical basis. Bacteriologists therefore resort to the more delicate criteria of protoplasmic structure and physiological activity, in which direction remarkable differentiation exists. Tests for the finer structural differences of these organisms are found in their behavior to differential stains, like the Gram stain; and to the immune substances induced by them in the animal body. Their physiological activity is measured by determining the end products of their metabolism. Bacteria generally have evolved in two main directions, one group possessing marked carbohydrate-splitting properties, the other having developed the property of digesting various protein substances. The streptococci belong to the former division, showing but little tendency to effect proteolysis.

It appears natural enough to assume that the biologic activities of a cell correspond with its protoplasmic constitution. Yet such a correlation has not been worked out except in a few isolated cases.

Among the streptococci such a correlation, if it exists, would be especially significant in that it would help to differentiate the various members of a genus that has puzzled many investigators.

The agglutination, fermentation and hemolytic properties of sixty strains derived from various pathological conditions were studied, using four agglutinating sera having a titre of 800-1000; and six carbohydrates and other fermentable substances as follows: Disaccharides—*lactose*, *sucrose*; trisaccharide—*raffinose*; alcohol—*mannite*; glucoside—*salicin*; polysaccharide—*inulin*.

Only twenty-seven of the strains were agglutinated by the sera used. A definite correlation was, however, obtained between the agglutinative and fermentative characters. The serum produced by a strain of one fermentative group (the group that fermented salicin, for instance) agglutinated only cultures of its particular division and failed to agglutinate members of any of the other groups. No such correlation was obtained with the hemolytic property, members of one hemolytic group being agglutinated by the sera produced by strains from another hemolytic group.

The results indicate that a separation of the streptococci obtained from various pathological conditions, into three fermentative types, would coincide most closely with their natural relationship. The groups suggested are:

(A). Salicin fermenters only, generally hemolytic.—*Str. pyogenes*.

(B). Raffinose fermenters; salicin usually fermented; mannite always negative; generally produces a green colony on blood agar.—*Str. salivarius*.

(C). Mannite fermenters; generally ferment salicin; rarely ferment raffinose; variable in their reaction to blood.—*Str. fecalis*.

163. The reducing power of anerobes. VICTOR E. LEVINE. (*Dep't of Public Health, Amer. Museum of Natural Hist., N. Y. City.*) It is a well established fact that anerobes reduce organic dyes, such as methylene blue (Cahen, Smith, Kitasato and Weyl). No difference on the ground of reduction may be claimed between aerobes and anerobes. Klett,¹⁴ however, using sodium selenite as an indicator, found that the anerobes he examined lacked the power

¹⁴ Klett: *Zeit. f. Hyg.*, 1900, xxxiii, pp. 135, 137.

of reduction. He also observed that sodium selenite, even in very small conc., inhibited growth, whereas sodium sulfite favored it. In the 1910 edition of Kruse's *Allgemeine Mikrobiologie*, the statement was made that anerobes do not seem to reduce sodium selenite, as indicated by the few preliminary findings of Klett.

In order to test the validity of this conclusion, experiments with sodium selenite were made with anerobes available in the bacteriological collection at the Museum of Natural History.¹⁵ The following organisms were used: *B. Welchi* (four strains); *B. sporogenes* (three strains); *B. Feseri* (two strains); *B. oedematis maligni* (two strains); *B. tetani* (two strains); *B. oedematis*; *B. botulinis*; *B. putrificus*. They were grown in media containing the following conc. of sodium selenite: 1 : 100,000; 1 : 50,000; 1 : 25,000; 1 : 10,000. The culture tubes were kept under anaerobic conditions by means of alkaline pyrogallate.

No appreciable inhibition of growth was observed except in conc. of 1 : 10,000. Reduction was found to have taken place within 24 to 48 hr. in conc. of 1 : 100,000, but the red selenium streak following the path of growth disappeared within a few days, so that there was no visible evidence of reduction. The higher selenite conc. showed excellent reduction but there was less tendency for the red precipitated selenium to disappear. At the end of 3 months the selenium streaks had completely disappeared in all the culture tubes except in the ones containing sodium selenite in conc. of 1 : 10,000.

These experiments prove conclusively that aerobes and anerobes reduce sodium selenite equally well and that sodium selenite cannot be used as a reagent for differentiation between these two classes of micro-organisms. For practical demonstrations, conc. of 1 : 25,000 and 1 : 10,000 yield the best results.

164. Body surface and metabolism of flounders. SERGIUS MORGULIS (*U. S. Fisheries Biological Station, Woods Hole, Mass.*) In connection with various biological problems the importance of the body surface has been frequently emphasized. Nevertheless, owing to the difficulties involved in measuring the surface of an organism, knowledge on this score has been very fragmentary. The flounder is an unusually favorable object for an investigation of

¹⁵ Levine : *BIOCHEM. BULL.*, 1914, iii, p. 464.

this matter. I have determined the surface of a large number of flounders ranging in size from about 4 to 25 cm.; and in weight, from about 0.5 to 150 gm. The surface can be computed from the formula $S = K\sqrt[3]{W^2}$, wherein W is the weight of the animal. K , which has been found to vary within narrow limits, is 13.44 for *normal* flounders. This value coincides closely with that found for higher organisms.

Under normal circumstances the metabolism, as judged by the oxygen consumption, diminishes per unit of body surface as the latter increases. The relation of the metabolism to surface was well illustrated, in a series of experiments, where the surface was reduced 30-40 percent by the removal of the fins. The body weight was very little affected by the operation, as the fins form only 2-3 percent of the weight. In this case the oxygen consumption remained unchanged, and must, therefore, have been dependent upon the mass of living substance.

Furthermore, it is important to bear in mind that the value of K is constant under definite physiological conditions. In fasting flounders the value of K has been invariably much higher. This was due to the fact that the body weight diminished more rapidly than the surface, and probably, also, because the specific gravity of the organism was decreased.

B. ABSTRACTS OF PAPERS FROM THE COLUMBIA BIOCHEM. DEPT

165. The neutral-sulfur and colloidal-nitrogen tests in the diagnosis of cancer.¹⁶ FREDERIC G. GOODRIDGE and MAX KAHN. Published in this issue: *BIOCHEM. BULL.*, 1915, iv, p. 118.

166. Sodium selenite as a laboratory reagent for reducing substances. VICTOR E. LEVINE. Further experiments confirm the statement¹⁷ that sodium selenite, in alkalin sol., can be used as an indicator for reducing substances, especially carbohydrates containing free carbonyl groups.

The following *do not reduce* sodium selenite (alkalin): acetone, formaldehyde, tri-oxy methylene, acetaldehyde, furool, benzaldehyde, cinnamic aldehyde, salicyl aldehyde, piperonal, methyl alcohol, ethyl

¹⁶ Some of the work was done in the Beth Israel Hospital, N. Y. City.

¹⁷ Levine: *BIOCHEM. BULL.*, 1913, ii, p. 552.

alcohol, glycerol, erythrol, mannite, inosite, phenol, the cresols, thymol, α -naphthol; acetic, butyric, β -oxybutyric, palmitic, stearic, trichloroacetic, oxalic, tartaric, citric, oleic, malic, cinnamic, and hippuric acids; glyocol, alanin, guanidin carbonate, leucin, urea, thio-urea, ammonium sulfocyanate, caffenin, theobromin, uric acid, sodium urate, creatinin, lecithin, cholesterol, palmitin, stearin, olein, serum proteins, blood fibrin, edestin, egg albumen, gelatin, peptone, proteoses, ovalbumen, collagen, osseomucoid, elastin, saccharin, anti-pyridin, anthraquinone, sucrose, raffinose, cellulose, starch, dextrin, glycogen, inulin, esculin, amygdalin, and the following gums: arabic, tragacanth, guaiac, rosin, benzoin, kino, aloes, asafetida, myrrh, gambir. Alcoholic solutions of gum benzoin, kino or aloes give a red-brown to cherry-red sol. without the addition of sodium selenite.

The following *reduce* sodium selenite: amidol, arabinose, rhamnose, xylose, glucose, galactose, fructose, maltose, lactose, hydroquinone, phloroglucinol, pyrogallol; hydroxylamin, phenylhydrazin and benzin hydrochlorids; hydrazin hydrate; arsenious, hydrobromic, hydriodic, phosphorous, hypophosphorous and sulfurous acids; ferrous sulfate, stannous chlorid, sodium thiosulfate, zinc and hydrochloric acid, hydrogen sulfid, acetylene; formic, gallic, lactic and tannic acids.

Acetone, acetaldehyde, formaldehyde, aceto-acetic ester, β -oxybutyric acid, creatinin, lactic acid, formic acid and inulin reduce in *acid*, but not in alkalin mixtures of sodium selenite. Methyl alcohol and ethyl alcohol reduce sodium selenite *strongly acidified* with sulfuric or with hydrochloric acid.

Oxalic, citric, tartaric, malic and salicylic acids, benzaldehyde, cinnamic aldehyde and salicyl aldehyde, reduce neither acid nor alkalin mixtures.

The results show that monosaccharids readily reduce alkalin sol. of sodium selenite. Pentoses effect readier and more profuse reduction than the hexoses and the reducing disaccharids. Of the pentoses, xylose causes the most striking reduction. Among the hexoses, fructose and galactose reduce more readily than glucose, and galactose less readily than fructose. Among the disaccharids only those having free carbonyl groups reduce. Maltose and lactose effect reduction, but sucrose does not; raffinose, cellulose, starch, dextrin, glycogen, inulin do not reduce.

In order to test the influence of acidity, or alkalinity, upon the reduction of sodium selenite, nineteen reagents were prepared. One consisted of sodium selenite neutralized with sulfuric acid. Ten were alkaline, the basicity being due to sodium selenite *per se*, to sodium bicarbonate, sodium carbonate, sodium tetraborate, sodium silicate, sodium hydroxid, di-sodium hydrogen phosphate, potassium hydroxid and Rochelle salt, or sodium carbonate and sodium citrate. Eight reagents were acidified by the addition of one of the following: potassium bi-sulfate, sodium di-hydrogen phosphate; hydrochloric, nitric, sulfuric, phosphoric, citric, or tartaric acid. When these reagents were heated none reduced, even with complete evaporation, except those containing citric or tartaric acid. These two reagents deteriorated after standing several months.

Experiments with the above-named reagents were conducted at 37.5° C. Solutions (0.5 percent) of arabinose, rhamnose, xylose, glucose, fructose, galactose, sucrose, maltose, lactose, glycogen, starch, dextrin, inulin, raffinose; mucic, lactic and formic acids; acetone, and formaldehyde, were tested. Three cc. of the sol. to be used were mixed with 2 cc. of the selenite reagent and toluene added. The tubes were kept at 37.5° C., and examined from time to time. Controls were run with Fehling and Fehling-Benedict reagents. The reagents containing sodium hydroxid and potassium hydroxid (selenite and Fehling) were the first to show reduction. Fehling reagent reduced more quickly than Fehling-Benedict. Glycogen, starch, dextrin, inulin and raffinose reduce acidified solutions of sodium selenite by the end of 4 days. Alkaline sol. were not affected. Formic acid, lactic acid, and formaldehyde reduce in acid sol. only. Acetone profusely reduces acid sol.; very faintly, some of the alkaline sol. The reagent, acidified with nitric acid, showed no reduction, except in the case of acetone. Neutralized sodium selenite is a very ineffective indicator of reduction. The presence of sodium tetraborate inhibits to a very striking extent the reduction of sodium selenite.

A sol. containing 2 percent of sodium selenite, 10 percent of sodium citrate, and 10 percent of sodium carbonate, has been tested with reducing sugars (100° C.). Reduction with this reagent takes place in one minute, or even less. At first a deep chlorine-yellow color is developed. After standing a minute or two, this color gives way to

a light, wine-red, tint, then to a dense brick-red precipitate, which suffuses the volume of the liquid. A 0.02 percent sol. of glucose causes fair reduction; in 0.01 percent sol., reduction is slight though perceptible. Sol. to be tested must be alkaline, and must not contain potassium cyanid or oxidizing agents (*e. g.*, free halogen, hydrogen peroxid, potassium permanganate, potassium bichromate). Sugar-free urine gives a positive reaction when it is acidified with hydrochloric acid. This positive reaction is probably due to acetone substances and creatinin, which reduce acidified sol. of sodium selenite.

Minute amounts of selenium, in the form of selenite ion, can be detected by a procedure similar to that of the Marsh test for arsenic. One mg. of selenium dioxid yields a characteristic dull red mirror, soluble in oxidizing agents.

167. A further study of the effects of acid media on natural extracted teeth. ALFRED P. LOTHROP and WILLIAM J. GIES, with the collaboration of HENRY W. GILLET, CHARLES C. LINTON, ARTHUR H. MERRITT and HERBERT L. WHEELER.¹⁸ The fermentation of glucose on normal and filled teeth, with sound enamel "worn very little or not at all," in the presence of saliva, induced rapid decalcification of the enamel and speedy disintegration of some of the fillings. These effects were more pronounced on salivated teeth covered with muslin than on similarly salivated teeth that were not thus covered.

Two daily brushings, for 4 months with tap water, and continuous daily treatment under muslin covers, during the intervening periods, with (a) water, (b) water containing carbon dioxid, and (c) water holding an abundance of salivary mucin (*mucin* not mucinate), failed to induce injurious effects on teeth with sound enamel "worn through and exposing dentin," and with enamel "worn very little or not at all."

Two daily brushings, for 4 months with tap water, and continuous daily treatment under muslin covers during the intervening periods, with (a) 0.25 percent solution of mono-sodium di-hydrogen phosphate (NaH_2PO_4), and with (b) aqueous suspension of salivary *mucin* (not mucinate) plus 0.25 percent solution of mono-sodium di-hydrogen phosphate, failed to induce injurious effects on either the enamel or the fillings in teeth having sound enamel that

¹⁸ *Journal of the Allied Dental Societies*, 1914, ix, p. 554.

was "worn very little or not at all," or which was "considerably worn without exposure of dentin."

Unfilled teeth, with sound enamel that was (a) "considerably worn without exposure of dentin," (b) "worn very little or not at all," or (c) "worn through and exposing dentin," when subjected to two daily brushings, in comparative tests, for 8 months, with (1) dilute vinegar (1:1), a (2) common tooth powder or a (3) common tooth paste, with intervening salivation, were uninjured. No change of any kind could be detected as a result of the vinegar treatment.

Two daily brushings of teeth similar to those referred to in the preceding paragraph (some of them filled), with dilute vinegar (1:1), for 8 months, 9 months and 17 months, were free from injurious influences on both the enamel and on most of the fillings,¹⁹ whether the teeth were salivated (covered or uncovered) during the intervening periods or not.

168. **The excretion of creatin during a fast.**²⁰ F. D. ZEMAN and PAUL E. HOWE. Recent criticism²¹ of results obtained with Folin's method for the determination of creatin in urine, in the presence of acetone and aceto-acetic acid, has thrown doubt upon the presence of creatin in the urine of fasting man. We have determined creatin in the urine of a fasting man throughout a 7-day fast. The method of Graham and Poulton was employed for the removal of acetone and aceto-acetic acid; quantitative determinations were made of these substances, together, and of β -hydroxy butyric acid. Control experiments were made with untreated urine. Determinations before and after the appearance of the interfering substances showed the method to be accurate in their absence. Creatin was excreted on each fasting day in amounts equal, in most cases, with those obtained in previous fasts under similar conditions.

169. **Recuperation: Nitrogen metabolism of a man when ingesting successively non-protein and normal diets after a**

¹⁹ The fillings consisted of the following materials: malleted gold, gold inlays, gutta percha, Ames' black copper cement, Stanley's red copper cement, Ames' pearl white inlay cement, Ames' beryllite, fellowship alloy, silicate cement, oxyphosphate of zinc, synthetic porcelain, alloy, amalgam.

²⁰ Most of the work was done in the Biochemical Laboratory at Teachers College.

²¹ Graham and Poulton: Proc. Roy. Soc., 1914, lxxxvii, B, p. 205.

seven-day fast.²² F. D. ZEMAN AND PAUL E. HOWE. The third²³ of a series of experiments on changes in metabolism of man following the ingestion of food after a fast. In the recuperation periods (4 days) of this experiment, non-protein and normal diets were fed; the preliminary and final diets were the same. The non-protein diet consisted of sucrose, clarified butter, alkaline salt mixture, and agar-agar, having an approximate daily fuel value of 3500 cal. Determinations were made of the body weight, and the excretion of urinary water, total nitrogen, urea, ammonia, creatin and creatinin.²⁴

The excretion of the *urinary* constituents followed the usual course during the fast; the total-nitrogen excretion on the 7th day was approximately 10 gm. and creatin appeared daily. The ingestion of a calorically sufficient, non-protein, diet resulted in decrease of the nitrogen excretion, which became constant on the 3d and 4th days. Minimum values obtained on the 2nd day of feeding, were as follows: Total N, 3.56 gm.; urea-N, 1.59 gm.; ammonia-N, 0.54 gm.; creatinin-N, 0.61 gm.; creatin-N, 0.05 gm. A relatively high ammonia-N excretion (0.72 gm., 17.4 percent of the total N) occurred on the 3d day. Normal conditions tended to return in the final period while the subject was retaining nitrogen. Lowered absolute and relative ammonia-N excretions were observed. The daily excretion of *fecal* nitrogen during the non-protein period was 0.50 gm.

A comparison of the changes in body weight, and in the nitrogen balances, shows an increase in body weight during the non-protein feeding period, accompanied by a loss of nitrogen; the reverse occurred in the final period. The initial increase in weight after the ingestion of food was the result, chiefly, of the retention of water and to a smaller degree of non-nitrogenous food substances.

170. Variations in factors associated with acidity of human urine, during a seven-day fast and during subsequent non-pro-

²² Most of the work was done in the Biochemical Laboratory at Teachers College.

²³ The first two experiments were reported by Howe, Mattill and Hawk: *Jour. Amer. Chem. Soc.*, 1911, xxxiii, p. 568, and Howe and Hawk: *Proc. Amer. Soc. Biol. Chem.*, 1912, ii, p. 65; *Jour. Biol. Chem.*, 1912, xi, p. xxxi.

²⁴ Variations in factors associated with changes in the urinary acidity are referred to in the succeeding abstract.

tein and normal feeding periods.²⁵ F. D. ZEMAN, JEROME KOHN and PAUL E. HOWE. A study was made of the variations in acidity (true and titratable) of human urine, with relation to modifying factors present during fasting and recuperation. The range of variations of the acidity extended from a fairly acid urine, $P_{\text{H}}^{\pm} 5.1$ (3rd day of fast) to an alkaline urine, $P_{\text{H}}^{\pm} 8.0$ (last day of the final period). The diet of the preliminary and final feeding periods was the same, in nature, as that used in previous experiments.²⁶ In the non-protein period, sucrose, clarified butter, salts (alkaline mixture) and agar-agar were ingested. Determinations were made of the H^+ ion conc. (indicators): titratable acidity or alkalinity (with phenolphthalein, neutral red and methyl orange); phosphates; ammonia; acetone-aceto-acetic acid; and β -hydroxy butyric acid.

In the absence of exogenous phosphorus (fasting) we found the acidity (true and titratable), phosphates, acetone-aceto-acetic acid and total nitrogen, varied together. During the non-protein, post-fasting, period there was an increased H^+ ion conc. and acidity, without accompanying increase in nitrogen excretion; acetone and aceto-acetic acid were absent. The increased excretion of ammonia in fasting is correlated with that of β -hydroxy butyric acid; when not influenced by this factor, as in the preliminary, non-protein, and final feeding periods, the ammonia excretion fluctuated with the H^+ ion conc. and the acidity. The low ammonia excretion in the final period showed that the low H^+ ion conc. and titratable acidity resulted from a loss of fixed base. This phenomenon is apparently characteristic of recuperation (nitrogen retention).

It seems probable that increased nitrogen excretion during the early days of a fast in a human individual is related to metabolic processes that result in the excretion of aceto-acetic acid.

III. TWENTIETH MEETING

The *twentieth scientific meeting* of the Assoc. was held in the Biochemical Seminar Room, at the Columbia Med. Sch., at 4:15

²⁵ Most of the work was done in the Biochemical Laboratory at Teachers College.

²⁶ Howe, Mattill and Hawk: *Jour. Amer. Chem. Soc.*, 1911, xxxiii, p. 568. Howe and Hawk: *Proc. Amer. Soc. Biol. Chem.*, 1912, xii, p. 65; *Jour. Biol. Chem.*, 1912, xi, p. xxxi.

P. M. on Feb. 5, 1915. The appended summary facilitates reference to the abstracts (171-176) of the papers presented, pages 224, 227.

A SUMMARY OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE SUCCEEDING ABSTRACTS (171-176)

A

ALLAN C. EUSTIS. The detoxicating effect of the liver of *Cathartes aura* upon solutions of β -imidazolyethylamin. (171)

V. E. LEVINE and HERMAN YAHR. Reductions with compounds of the rarer elements: I. Ammonium molybdate. (172)

MAX MORSE. Autolysis and nuclear relations. (173)

EDWIN D. WATKINS. Studies of some compounds of cinchona alkaloids, certain metals and phosphoric acid. (174)

B

F. G. GOODRIDGE. Biochemical studies of mercaptan. (175)

M. K. THORNTON. Efforts to precipitate pepsin and erepsin with safranin. (176)

A. ABSTRACTS OF PAPERS BY NON-RESIDENT MEMBERS

171. The detoxicating effect of the liver of *Cathartes aura* upon solutions of β -imidazolyethylamin. ALLAN C. EUSTIS. (*Dept of Dietetics and Nutrition, Coll. of Med., Tulane Univ., New Orleans.*) Published in this issue: *BIOCHEM. BULL.*, 1915, iv, p. 97.

172. Reductions with compounds of the rarer elements. I. Ammonium molybdate. VICTOR E. LEVINE and HERMAN M. YAHR. (*Lab. of Organic Chem., Fordham Univ. Med. Coll., N. Y. City.*) Ammonium molybdate, in acid sol., heated with many organic compounds, gives rise to a green, greenish-blue, or blue coloration. That this effect is due to reduction of the ammonium molybdate may be concluded from the following observations. Gaseous hydrogen produces the characteristic color, when led through a sol. of the molybdate acidified with hydrochloric or preferably with sulfuric. Acetylene, sulfur dioxid and hydrogen sulfid react similarly. Carbon mon-oxid gives negative results. Potassium iodid treated cold, and sodium bromid treated hot, with ammonium molybdate acidified with sulfuric acid, also produce a blue color. Ferrous and stannous compounds can be distinguished from ferric and stannic, the former two giving the color reaction; the latter, not. Arsenious oxid yields a dark green color. Oxidizing

agents (hydrogen peroxid, sodium peroxid, nitric acid, potassium nitrate, manganese dioxid, potassium chlorate, potassium di-chromate, potassium permanganate) destroy the color or inhibit its formation. Furthermore, the color sometimes fades when the colored sol. exposed to the air is allowed to stand.

Many organic substances reduce acidified sol. of ammonium molybdate. Sulfuric acid gives far better results than hydrochloric acid; phosphoric and nitric acids should not be used. To determine the influence of the conc. of sulfuric acid upon the intensity of the reduction three reagents were prepared. *Reagent A* consisted of 30 gm. of ammonium molybdate, and 25 cc. of conc. sulfuric acid in 1 l. of dist. water. *Reagent B* contained the same amount of molybdate, but 50 cc. of the acid per l. *Reagent C* contained 100 cc. of the acid per l. The sol. to be tested were heated in a water-bath for from 5 to 30 min. It was found that *Reagent A* yielded the most intense reductions, *Reagent B* gave weaker colors, while *Reagent C* gave negative results in most cases. The greater the conc. of sulfuric acid, the less sensitive the reagent.

Many compounds gave strikingly beautiful reactions. These are fructose and the carbohydrates that yield it by hydrolysis (sucrose, raffinose, inulin), rhamnose, arabinose, xylose, maltose, starch, glycogen, dextrin, agar-agar, amygdalin, salicin, esculin; the gums—benzoin, tragacanth, gambir, asafetida, guaiac, myrrh, kino and acacia; mucic acid, formaldehyde, trioxymethylene, acetaldehyde, acetaldehyde-ammonia, reduced oxalic acid, erythrol, resorcinol, tricresol, hydroquinon, orcinol, tartaric acid, malic acid, tannic acid, amidol; phenylhydrazin (cold), *p*-phenylene-diamin, benzidin and hydroxylamin hydrochlorids; hydrazin sulfate, caseinogen, osseomuroid and thiourea. *Less intense* reduction was observed with glucose, arbutin, mannite, aceto-acetic ester, chloral, benzaldehyd, *p*-nitrobenzaldehyd, di-methyl aminobenzaldehyd, cinnamic aldehyd, salicylaldehyd, cumarin, acetone, *p*-amidoacetophenon, methyl alcohol, ethyl alcohol, glycerol, phenol, *m*- and *p*-cresols, thymol, α -naphthol, phloroglucinol; oxalic, citric, gallic, lactic, and uric acids; caffein, salicylic acid, asparagin, leucin, edestin, egg albumen, fibrin, collagen, gelatin, proteoses, serum protein, mucoid, ovalbumin, creatinin zinc chlorid, nitrobenzol, lecithin, choles-

terol, olive oil, olein, palmitin, stearin. *Still weaker* reductions were obtained with vanillin, hippuric acid, *p*-amino aceto-phenol, chloretone and camphor oxim. Benzene sodium sulfonate, potassium ethyl sulfate, palmitic and stearic acids, and urea, gave *negative* results. Potassium sulfocyanate yielded a red coloration with *Reagent A*. On diluting with water the liquid became greenish blue. When minute amounts of the sulfocyanate were used, the characteristic green or blue color, indicative of reduction, was observed at once.

Miller and Taylor²⁷ found that acetone, acetaldehyd, benzaldehyd, vanillin, glycerol, phenol, thymol, orcinol, phloroglucinol, salicylic acid, uric acid and tannic acid failed to reduce ammonium molybdate. They observed that although ketones and aldehydes did not reduce, ketone and aldehyde sugars did reduce. Our findings are not in accord with those of Miller and Taylor quoted above. Aldehydes, ketones, monosaccharids, disaccharids, polysaccharids, gums, glucosides and glucoproteins and also other proteins reduced acidified sol. of ammonium molybdate. Egg albumen reduced even in the cold. Glucose reduced slightly in comparison with fructose. Lecithin, olein, palmitin and stearin reduced, owing, probably, to the presence of the glyceryl radical, glycerol itself causing reduction. Phenol reduced even in cold acetic or sulfuric acid mixture of ammonium molybdate. Uric acid gave positive results.

The reactivity of ammonium molybdate in this respect is too general to be of value as a differential test. The study is in progress.

173. Autolysis and nuclear relations. MAX MORSE. (*Dep't of Physiol., Univ. of Wis., Madison.*) There is a more or less direct relation between the character of an organ with respect to its nuclear content, from the histological standpoint, and its rate of autolysis, whereby those organs in which there are relatively greater masses of nuclei to that of matrices (cytoplasm, interstitial substance, protoplasmic differentiation in the form of fibres, etc.), such as glands, show greater rates of Salkowskian autolysis. Accordingly, the hypothesis might be formulated that some connection exists between the distinctive chemical component, nucleic acid, and the rate of tissue-enzyme action. This hypothesis was tested by

²⁷ Miller and Taylor: *Jour. Biol. Chem.*, 1914, xvii, p. 531.

adding given amounts of liver, spleen and thymus nucleic acid²⁸ to pig liver *brei*, and following the rate of autolysis by estimations of total nitrogen on tannic acid filtrates.

As the accompanying table shows, there was no apparent modification of rate of enzyme action. Doubtless the relation between nuclear component and rate of autolysis concerns the activity of organs which are relatively richer in nuclei, such organs being in a more active state, metabolically.

Table showing relation between percentage of thymus nucleic acid and rate of autolysis in pig liver *brei* as measured in terms of c.c. of $n/5$ NH_3 for 25 c.c. aliquot portions of tannic acid filtrates

Percent of sodium nucleate	Initial	24 hr.	2 days	6 days	9 days	12 days
Control	1.2	3.2	3.4	4.2	4.5	5.1
0.5	1.1	3.3	3.7	4.0	4.4	4.4
1.0	1.3	3.5	4.5	4.9	5.3	5.7
2.0	1.7	2.9	4.0	4.1	4.7	4.5
5.0	2.3	2.3	4.0	4.6	5.1	5.0

174. Studies of some compounds of cinchona alkaloids, certain metals and phosphoric acid. EDWIN D. WATKINS. (*Univ. of Tenn., Memphis.*) Published in this issue: *BIOCHEM. BULL.*, 1915, iv, p. 94.

B. ABSTRACTS OF PAPERS FROM THE COLUMBIA BIOCHEM. DEPT

175. Biochemical studies of mercaptan. F. G. GOODRIDGE. Mercaptan, when given subcutaneously to either cold or warm blooded animals, has marked anesthetic effects. The first result of the administration is irritation, and then follow promptly abolished reflexes and loss of consciousness. Respiration is at first increased and then slowed. The heart is rapid and feeble and, in warm blooded animals, the temperature is much reduced, and the color of the blood is changed to a dark brown. If the elimination by means of the breath is not prompt and thorough, the kidneys become impaired, and acute parenchymatous nephritis supervenes. This condition causes death after an interval of from one to five days. When death follows promptly after the administration, it is probably due to respiratory depression.

²⁸ Jones and others have shown that all animal nucleic acids are undoubtedly identical, chemically.

Inhalation of mercaptan causes rapid and overwhelming results. Anesthesia is complete in less than a minute and, if the animal is not promptly exposed to the air, death follows quickly from respiratory depression.

The administration of the drug *per os* causes nausea, vomiting and increased peristalsis. There is irritation and impairment of the kidneys, and these organs are rendered more permeable to the passage of glucose. This damage, as shown by the urinary findings, rapidly passes off and the kidneys return to normal.

176. **Efforts to precipitate pepsin and erepsin with safranin.** M. K. THORNTON. Neither pepsin nor erepsin (unlike trypsin) was precipitated by safranin from gastric and intestinal extracts; or, if they were precipitated under the conditions of the tests, the products were inactive. The experiments are in progress.

OFFICERS ELECTED AT THE EIGHTEENTH MEETING²⁹

HONORARY OFFICERS. President—Prof. *Alfred P. Lothrop*, Queens University, Kingston, Ont. Vice-presidents—Prof. *John S. Adriaance*, Williams College; Prof. *Josephine T. Berry*, University of Minnesota; Dr. *E. Newton Harvey*, Princeton University; Prof. *Burton E. Livingston*, Johns Hopkins University; Mrs. *Jessie Moore Rahe*, Cornell University Medical College.

ACTIVE OFFICERS. President—Prof. *A. J. Goldfarb*; vice-president, Dr. *Alfred F. Hess*; secretary, Dr. *Edgar G. Miller, Jr.*;³⁰ treasurer, Prof. *William J. Gies*. Additional members of the executive committee—Dr. *F. G. Goodridge*, Prof. *Paul E. Howe*, Dr. *William Weinberger*.

²⁹ See page 193.

³⁰ Elected at the nineteenth meeting. See page 210.

*Biochemical Laboratory of Columbia University,
College of Physicians and Surgeons,
New York.*

BIOCHEMICAL BIBLIOGRAPHY AND INDEX

8-10. Third and fourth quarters, 1914 (July-Dec.); and first quarter, 1915 (Jan.-Mar.)

WILLIAM A. PERLZWEIG AND WILLIAM J. GIES

(*Biochemical Laboratory of Columbia University, at the College of Physicians and Surgeons, New York*)

Change in the plan of presentation. Publication of the current portions of our "biochemical bibliography and index"¹ has been interrupted because of unavoidable delay in the issuance of this number of the BIOCHEMICAL BULLETIN (page 270). The European war has also affected the bibliography and index, by reducing materially the output of papers in, and numbers of, the European publications on our journal list. This delay in publication, and the simultaneous curtailment of content, have suggested an improved plan for the presentation of the bibliography and index, which we inaugurate herewith.

The chief improvement consists in the arrangement of titles in subject-groups rather than, as heretofore, in the order of the placement of the papers in the successive numbers of the respective journals. Each title is placed under the subject-head that is suggested by the *main* feature of the *content* of the corresponding paper. Thus, papers consisting primarily of descriptions of *methods* are indicated collectively under "Methods." This arrangement follows the general style of that proposed by the senior author some years ago for the Biochemical Department of *Chemical Abstracts*, and which is still in vogue. The advantages of this arrangement are so obvious that a mere statement of its adoption here is sufficient to suggest its purpose and its merits.

Considerable space is saved by the use of small letters to indicate particular volumes of the listed journals. The position and significance of these letters are indicated below.

The names of authors *follow*, in this new plan, the titles of the papers, whereas, in the preceding issues, the reverse order was the rule.

The bibliography and index remain practically unchanged otherwise, both in style and scope.

¹ Gies: *BIOCHEM. BULL.*, 1913, ii, pp. 298, 470, 559; Perlzweig: *Ibid.*, 1913, iii, pp. 103, 315, 475.

[Volumes. **BZ**: a=65; b=66; c=67; d=68. **ZpC**: g=92; h=93. **JBC**: m=18; n=19; o=20. **BJ**: s=8. **JACS**: w=36; x=37. **BB**: y=3. (See page 230.)]

Explanation of abbreviations, arrangement, notation, etc. BIBLIOGRAPHY. *Titles of papers* are freely shortened, minor words ignored, common terms conveniently abbreviated or chemical symbols substituted; *surnames* of collaborators are connected by hyphens; most *punctuation marks* are omitted—all for the sake of condensation. Names of authors are printed in italics. *Bibliographic items* are separated by em dashes, and are preceded by numerals indicating, for index purposes, sequence in the bibliography. When two or more papers by the same author occur together, they are regularly numbered and separated by semicolons; but follow one em dash. *The numeral at the end of each item*, separated from the name of the author or authors by a comma and a small letter, indicates *initial page of the corresponding paper*. *The small letters between the names of the authors and the page numerals*, at the ends of the items, represent *specific volumes of the listed journals*, as indicated below:

*Biochemische Zeitschrift (BZ)*²: a=65; b=66; c=67; d=68.

Zeitschrift für physiologische Chemie (ZpC): g=92; h=93.

Journal of Biological Chemistry (JBC): m=18; n=19; o=20.

Biochemical Journal (BJ): s=8.

*Journal of the American Chemical Society (JACS)*³: w=36; x=37.

Biochemical Bulletin (BB): y=3.

INDEX (SUBJECTS). The numerals in the index (page 239) correspond with the numbered items in the bibliography. *Pages are not indicated*. Numerals held in groups by hyphens are plain abbreviations in accord with the indications of the first numeral of each such series (see footnote, p. 239). Abbreviations of words in the index are similar to those in the bibliography. Each *group of index references* is terminated by a semicolon; commas mark off *subdivisions of a general index subject*. *Names of authors are not indexed*.

PRACTICAL USE OF THE BIBLIOGRAPHY. The bibliography is useful from several standpoints. Thus, if it is desired to ascertain whether the journals included in the bibliography contain papers (during the indicated period) on a particular subject, *e. g.*, alcohol, find the key word in its alphabetic place in the index and turn to the items in the bibliographic sequence indicated by the index numerals [in this case 178, 180, 181, 183, 186, 205, 342, 351, 365]. The abbreviated items thus identified give the names of authors and suggest the nature of the corresponding papers (nine papers in the case selected for illustration), and help the reader to decide whether to examine the original publications. When the index gives a negative answer to an inquiry, a large mass of literature in that connection is removed from further consideration. During the intervals between publication of the indexes of journals, *Zentralblätter* and year books, this running bibliography directs the reader to the main tracks through most of the current literature on the leading biochemical subjects.

² Abbreviations employed at the tops of the pages.

³ Included in this bibliography for the first time. The section in the *Journ. Amer. Chem. Soc.* regularly devoted to biological chemistry is steadily growing in interest and importance.

[Volumes. **BZ**: a = 65; b = 66; c = 67; d = 68. **ZpC**: g = 92; h = 93. **JBC**: m = 18; n = 19; o = 20. **BJ**: s = 8. **JACS**: w = 36; x = 37. **BB**: y = 3. (See page 230.)]

Apparatus.—1 Bericht'g: Mik' resp'nsapp, *Krogh*, b512.—2 Polaris'app Mik- u Mak'bestim b weis Licht, *Neuberg*, c102.—3 Resp'n app small anim, *Benedict*, o301.—4 Simp quartz Hg-vapor lamp, biol a photochem invest, *Bovie*, o315.—5 Easy calorim m high precis, *White*, w2313.—6 Modif Kjeldahl flask, soil N, *Noyes*, w2541.

Methods.—7 "Lackmosol," empfind Bestand Lacmoid, *Hottinger*, a177.—8 Manomet M Harnst'bestim, *Löw-Prorok*, a273.—9 Kol'd N Harn; Bedeut Carcinomdiag, *de Bloeme-Swart-Terwen*, a345.—10 Anal Ca Kot u Harn, *derHeide*, a363.—11 Nachw Peptid i Harn mit p-Kresol-Tyrosinas-Reak, *Chodat-Kummer*, a392.—12 Tryptophanbestim i Nier, *Kurchin*, a451.—13 Refrakt- u Dispers'bestim Fet u Öl, *Scalagyi*, b149.—14 Stalagmon Bestim klein OH-konz, *Gröh-Götz*, b165.—15 Stalagmon Studien kryst u kol'd Lös (5-10), *Berczeller*, b173.—16 Entsteh u Spezif Blütferm b Anwend Abderhalden Dial'verfahren, *Parsanow*, b269.—17 Triketohyd'reak, *Neuberg*, c56.—18 Fäll Am'säu mit Hg-acet u Soda, *Neuberg-Kerb*, c119.—19 Ninhyd'reak Pepton; Ferm u Antifer'm'wirk Serum, *Fränkel*, c298.—20 M Lecithinbestim Milch, *Brodrick-Pittard*, c382.—21 Wassermann Reak norm Mensch'serum, *Krauss*, d48.—22 Quant Bestim Milchsäu in Organextr als CO, *Meissner*, d175.—23 Formald'reak, *Salkowski*, d337.—24 Darstel indox-H₂SO₄ (Indican), *Jolles Schwenk*, d347.—25 Asym Synth Mandelsäu; Entsteh Benzylid'weinsäu'est u Benzylid'weinsäu, *Erlenmeyer*, d351.—26 Zerstör org Subst n Meth Fresenius-Babo, b vorher Behand mit Antiform u Bestim klein Pb-meng so behand Organ, *Friedmann*, g46.—27 Bestim Glykog in Hefe, *Salkowski*, g75.—28 Extrak'tof Muskeln: (16) Isol Carnosin dur Hg-ox'sulf, *Dietrich*, g212.—29 Fleischextrakt, *Smorodinzew*, g214.—30 Gewin Carnosin aus b Steriliz Fleisch m H₂O-dampf i Hönnecke-Fleischdampf sich bild Brüh, *Smorodinzew*, g228.—31 Quant Bestim Hg Harn, *Klotz*, g286.—32 Lactacidogen: (1) Isol, *Embden-Lacquer*, h94.—33 Ureas Sojabohn u Verwend quant Harnst'bestim, *Eigenberger*, h370.—34 Creatin a cr'inin metab: (1) Prep creatin a cr'inin urin, *Benedict*, m183.—35 *Idem*: (2) Est creatin, *Benedict* m191.—36 Est P in biol mater, *Taylor-Miller*, m215.—37 Turbid m determ acetone, acetoacet acid and β-oxybutyr acid urin, *Folin-Denis*, m263.—38 Quant determ album urin, *Folin-Denis*, m273.—39 Vol m tot S urin, *Raiziss-Dubin*, m297.—40 Purif a melt point sat aliphatic acid, *Levene-West*, m463.—41 Simp m determ tot N colorim, *Gullick*, m541.—42 Dry urin chem anal, *Braman*, n105.—43 Prep ureas, determ urea, *VanSlyke-Cullen*, n211.—44 Isol subst butter fat exerts stim infl grow, *McCollum-Davis*, n245.—45 Determ I connect thyr'd activ, *Kendall*, n251.—46 Determ sugar bl'd, *Shaffer*, n285.—47 Catal react bl'd: (1) Factors benzid test occult bl'd, *Lyle-Curtman-Marshall*, n445.—48 Est fat feces, *Gephart-Csonka*, n521.—49 Precip serum-alb a glutin by alk'oid reag, *Hanzlik*, o13.—50 Folin-Farmer m colorim estim N, *Bock-Benedict*, o47.—51 M sugar small quant bl'd, *Lewis-Benedict*, o61.—52 M inorg PO₄ tissue a food product, *Chapin-Powick*, o97.—53 Est benzoic acid urin, *Raiziss-Dubin*, o125.—54 Use col'd Fe determ lactos milk, *Hill*, o175.—55 Colorim m am'acid N, *Harding-MacLean*, o217.—56 M groups in P-lipin, *Foster*, o403.—57 Correct: M inorg PO₄ tissue a food product, *Chapin-Powick*, o461.—58 M est sugar bl'd, *Gardner-Maclean*, s391.—59 Produc ω-hydrox-s-meth'furfurald fr carbohydrate a infl on est pentosan a meth'-pentosan, *Cunningham-Dorice*, s438.—

[Volumes. **BZ**: a = 65; b = 66; c = 67; d = 68. **ZpC**: g = 92; h = 93. **JBC**: m = 18; n = 19; o = 20. **BJ**: s = 8. **JACS**: w = 36; x = 37. **BB**: y = 3. (See page 230.)]

60Quant est aspart a glutam acid, prod prot hydrol,*Foreman*,s463.—61Regulat mixt, recent indicator (2),*Walpole*,s628.—62Est allantoin urin presence glucos, *Plimmer-Skelton*,s641.—63Grav est minute quant P,*Raper*,s649.—64Determ As org mat,*Vinograd*,w1548.—65Invers sucros invertas:(8)M prepar strong invert sol fr top or bot yeast,*Hudson*,w1566.—66Adsorp glucos bone-black,*Morton*, w1832.—67Nat'l indicator,*Brubaker*,w1925.—68Prep raffinof,*Hudson-Harding*, w2110.—69Compar m determ proteol activ pancr prep,*Long-Barton*,w2151.—70 Nat'l indicator,*Brubaker*,w2385.—71Sol cryst dl-glycer ald fr syrup by oxid glycerol,*Witzemann*,w2223.—72Quant extr diastas fr plant tissue,*Thatcher-Koch*, w2542.—73Sep constit nat'l gas fr which gasolin condens,*Burrell-Seibert*,x392.—74Determ small quant HCN,*Viehoever-Johns*,x601.—75Quant determ S pepton, *Redfield-Huckle*,x607.—76Amylas:(9) Purif malt amyl,*Sherman-Schlesinger*,x643.—77Determ S cult med detec bacter produc H₂S,*Redfield-Huckle*,x612.—78Abderhalden serum test pregn,*Rosenbloom*,y373.—79Purif antigen Besredka serum diag tuberc,*Bronfenbrenner-Rockman*,y375.—80Diag value Landau test syph, *Bronfenbrenner-Rockman*,y377.—81So-called protect ferm:(1)Sensitiz substr Abderhalden test.*Bronfenbrenner-Mitchell-Schlesinger*,y386.—82Stand determ NH₃ Nessler sol,*Rose-Coleman*,y407.—83Mic'-ureas determ urea, *Rose-Coleman*, y411.

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⁴ See also "Nutrition (abnormal)" and "Immunity."

⁵ See also "Fermentation" and "Bacteria (fungi)."

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* This series of abbreviations, illustrating all others in the index, represents the following sequence of numerals: 178, 405, 406.

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BIOCHEMICAL NEWS, NOTES AND COMMENT

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I. GENERAL

Necrology.—*Angelo Celli*, prof. of hygiene, Univ. of Rome, chief of the Nat'l Board of Health, Italy, and senator.—*Wm. L. Dudley*, dean and prof. of chem., Vanderbilt Univ.—*Rudolph Emerich*, prof. of hygiene and bacteriol., Univ. of Munich.—*L. von Frankl-Hochwart*, prof. of path. of the nervous system, Univ. of Vienna, pioneer in research in neuropathol., internal secretions, etc.—*W. H. Gaskell*, univ. lecturer in physiol., Cambridge Univ.—*Karl Liebermann*, prof. of organic chem., Berlin.—*T. Wesley Mills*, emeritus prof. of physiol., McGill Univ.—*G. R. Mines*, prof. of physiol., McGill Univ.—*Chas. S. Minot*, prof. of comp. anat., Harvard Univ.—*Geo. L. Peabody*, prof. of materia med. and therap., Columbia Univ. (1887–1903).—*Charles Périer*, pres. French Acad. of Med.—*J. Rosenthal*, prof. of physiol., Erlangen.—*Daniel E. Salmon*, first chief, U. S. Bur. of Animal Industry (1884–1906).

Resignations and retirements. Barnard Free Skin and Cancer Hosp. (St. Louis): Dr. *Leo Loeb*, pathologist.

Carnegie Institution: Dr. *Simon Flexner*, trustee.

N. Y. Univ. and Bellevue Hosp. Med. Coll.: Dr. *W. H. Park*, dean; to devote all his time to his duties as Director of Laboratories, N. Y. Dep't of Health.

U. S. Dep't of Agric., Bur. of Chem., Food Research Lab. (Phila.): *A. D. Greenlee*, assis. chemist, to do commercial work.

Univ. of Geneva: Dr. *J. L. Prevost*, prof. of physiol.

Univ. of Liverpool: Dr. *Benjamin Moore*, prof. of biochem.

Univ. of Munich: Dr. *A. von Baeyer*, prof. of chem.

Univ. of Oregon: Dr. *J. M. Conolly*, prof. of physiol. chem.

West Va. Univ.: Prof. *E. D. Sanderson*, dean of the Coll. of Agric. and director of the Agric. Exp. Sta'n.

Appointments.¹ Baylor Univ. (Dallas, Texas): *Maxwell Sillman* (instr. in physiol. chem., Jefferson Med. Coll.), instr. in chem.

Dartmouth Coll.: Dr. *W. L. Mendenhall* (Drake Univ.), assis. prof. of pharmacol.

General Memorial Hosp. (N. Y.): Dr. *Casimir Funk* (Cancer Hosp. Research Inst., London), investigator of problems involving cancer and beri-beri, viewed from the standpoint of vitamins.

Harvard Univ.; Dr. *Roger I. Lee*, prof. of hygiene.

Jefferson Med. Coll., Dep't of Physiol. Chem. and Toxicol.: *J. O. Halverson*, instr.; *R. J. Miller*, instr.; Dr. *M. E. Rehfuess*, research assoc.; Dr. *Olaf Bergeim*, demonstrator (promotion).

Kiel Univ.: Dr. *Rudolf Höber*, prof. of physiol.

New Haven Hosp., Med. Board: Dr. *Frank P. Underhill*, chemist.

Panama Exp. Sta'n: Dr. *B. H. A. Groth* (plant physiologist, N. J. Coll. Exp. Sta'n), director.

Penn. State Coll., Inst. of Animal Nutrition: Dr. *F. C. Dosé*, assis. in animal nutrition.

Rockefeller Inst. for Med. Research: Prof. *Theobald Smith* (prof. of comparative pathol., Harvard Univ.), member.

Royal Institution: Dr. *C. S. Sherrington*, Fullerian prof. of physiol.

Toronto Univ.: Dr. *A. B. Macallum, Jr.*, instr. in physiol. chem.

Tulane Univ., Sch. of Hygiene and Tropical Med.: Dr. *A. L. Metz*, prof. of chem.

U. S. Dep't of Agric., Bur. of Chem.—Drug Inspection Lab. (Cincinnati, O.): Dr. *J. M. Humble* (Food Research Lab., Phila.),

¹ In this summary institutions from which appointments were made are named in parenthesis. See also pages 257 and 267.

assis. chemist; Food Research Lab. (Phila.): Dr. *C. M. Sherwood* (U. S. Public Health Serv.), bacteriologist; *L. H. Almy* (Bur. of Chem., Wash.), assis. chemist in fish-handling studies; *H. L. Shrader*, investigator in poultry- and egg-handling. Bureau of Plant Ind.: Prof. *G. R. Lyman* (Dartmouth Coll.), plant pathologist.

U. S. Public Health Service (Spartanburg, S. C.): Dr. *J. R. Murlin* (Cornell Univ. Med. Coll.) has leave of absence to serve as biochemist at the Pellagra Hosp.

Univ. of Ark., Coll. of Agric.: Mr. *De Forest Hungerford* (instr. in soils, Univ. Minn.), assis. prof. of agronomy.

Univ. of Calif.: Dr. *H. S. Reed* (plant pathologist and bacteriologist, Va. Agric. Exp. Sta'n, and prof. of mycol. and bacteriol., Univ. of Va.), prof. of plant pathol., at the Grad. Sch. of Tropical Agric., Riverside; Hooper Foundation of Med. Research: Dr. *R. H. Kocher*, instr. in research med., Dr. *G. H. Whipple* (Johns Hopkins Med. Sch.), director.

Univ. of Ill.: Dr. *A. O. Shaklee*, assoc. prof. of pharmacol.

Univ. of Liverpool: Dr. *Walter Ramsden* (Oxford Univ.), Johnston prof. of biochem.

Univ. of London, London Hosp. Med. Coll.: Dr. *E. P. Cathcart* (Glasgow Univ.), prof. of physiol.

Univ. of Munich: Dr. *F. v. Müller* (prof. of med.), rector of the Univ., 1914-15.

Univ. of Neb., Coll. of Med.: Dr. *J. D. Pilcher* (West. Reserve Univ., Med. Sch.), assoc. prof. of pharmacol.

Univ. of Oregon, Med. Sch.: Dr. *H. D. Haskins* (West. Reserve Univ., Med. Sch.), prof. of biochem.

Univ. of Penn., Sch. of Med.: Dr. *A. I. Ringer*, assis. prof. of physiol. chem. (promotion). [The phrase "Dental Sch." at the end of this item on p. 492 of Vol. III was a typographical error.]

Univ. of the Philippines: Dr. *H. G. Deming* (Philippine Coll. of Agric.), prof. of chem. and chief of the dep't.

Univ. of Pittsburgh, Mellon Inst. of Industr. Research: Prof. *M. A. Rosanoff* (Clark Univ.), prof. of chem. research.

Univ. of Sheffield: Prof. *J. B. Leathes* (Univ. of Toronto), prof. of physiol.

Univ. of Toronto: Dr. *Andrew Hunter* (formerly assis. prof. of biochem., Cornell Univ., recently biochemist, U. S. Public Health Service), prof. of pathol. chem.

Univ. of Wis.: Prof. *S. F. Acree* (Johns Hopkins Univ.), chief of

the Sect. of Derived Products, in the Forest-Products Lab., Madison; and prof. of the chem. of forest products.

West. Reserve Univ., Med. Sch.: Dr. *G. E. Simpson*, instr. in organ. chem. and demonstr. of biochem.

Honors. HONORARY DEGREES. Dr. *Simon Flexner*, Sc.D., Brown Univ.; on the occasion of the 150th anniv. of its foundation.—Dr. *E. W. Hilgard* (1874–1906, prof. of agric. and dean, Coll. of Agric., Univ. Cal.), LL.D., Univ. of Cal.—Conferred at Charter Day exercises, Univ. of Pittsburgh: Feb. 26; LL.D.: Prof's *Ira Remsen*, *T. W. Richards*, *E. W. Morley*, *A. A. Noyes* and *J. U. Nef*; D.Sc.: *J. J. Abel*; D.Ch.: *E. F. Smith*, and Dr. *Chas. L. Parsons*.

DINNERS. Prof. *W. B. Cannon* was guest of honor at a banquet following the ann. meeting of the Syracuse Univ. Chapter, Alpha Omega Alpha Fraternity, March 18. He delivered an address on The psychology of martial emotions.—The past and present members of the staff of the Rockefeller Inst. for Med. Research gave a dinner, at Delmonico's, to Dr. *Simon Flexner*, Oct. 16, '14 in celebration of the 10th anniv. of the opening of the laboratories of the Inst. under his direction.—A complimentary dinner was given, Jan. 14, at the Chemists's Club (N. Y.), in honor of Dr. *C. A. Mayo*, Pres't of the Amer. Pharmaceut. Assoc.—Dr. *S. J. Meltzer* was the guest of honor at the 4th ann. dinner of the Columbia Univ. Biochem. Assoc. (See page 261).—Dr. *Harvey W. Wiley* was the guest of honor at a dinner of the Twilight Club, at the Hotel McAlpin, N. Y., Jan. 9. Drs. Haven Emerson and Woods Hutchinson were among the speakers.

JOURNALISTIC. Vol. 37 of the *Amer. Jour. of Physiol.* has been dedicated to Prof. Wm. T. Porter, in terms of the following tribute, which appears with Dr. Porter's portrait in the first number (April issue):

“TO WILLIAM TOWNSEND PORTER: When physiological science in America was searching for a suitable medium for the publication of the increasing output of its laboratories and when no solution of the vexing problem seemed at hand, *William Townsend Porter* proposed the establishment of a new journal, to be called *The American Journal of Physiology*, and offered to undertake its administration. The American Physiological Society contributed its name and its

moral support; Professor Porter took upon himself the editorial and the financial burdens. These he has borne through sixteen years and through the *Journal's* first thirty-three volumes. From its inception his ideals were high. He believed that a meritorious discovery may fail of appreciation because of the faulty manner in which it is announced to the world, and that an editor may be of service to an investigator. He believed that a scientific journal, the organ of a national science, should be characterised by scientific merit, rhetorical excellence, the prompt publication of its contributions, and typography and illustration that are pleasing to the eye. These ideals he has maintained. A rigid pursuit of ideals by one individual frequently arouses in others lack of appreciation, criticism, and opposition; and these he has received without complaint. Time and effort and sacrifice of personal considerations have been given by him without stint. In now laying down these burdens and generously transferring to the American Physiological Society his interests he has given over a journal that has an assured position of merit among journals of physiology and that has been one of the chief agencies in the unification of American physiology. For his unselfish labors Professor Porter deserves the thanks of American physiologists and as an expression of this gratitude they gladly dedicate to him this volume."

A recent number of the *Zeitschrift für Untersuchung der Nahrungs- und Genussmittel* has been issued in honor of Dr. Joseph König, prof. of hygiene and food chem., Münster, in celebration of the 70th anniv. of his birth. The number consists wholly of papers by some of König's pupils.

Prof. Élie Metchnikoff, assis. director of the Inst. Pasteur, will soon celebrate his 70th birthday and the 50th anniv. of his doctorate. A commit. has been formed, under the presidency of Dr. Roux, director of the Inst. Pasteur, for the celebration of the anniv., which will include the publication of a "Festschrift."

Lectures and addresses. ENDOWED LECTURES. Coll. of Phys., Phila.; *Mütter Lect.*, Dec. 4, '14: Prof. F. H. Albee, The fundamental principles involved in the use of the bone graft in surgery.

Columbia Univ.; *Phi Lambda Upsilon Lect.*, Dec. 10, '14 Prof. H. A. Huston (German Kali Works), The potash industry.

N. Y. Acad. Med.; *Harvey (Society) Lect.* of biochemical interest—Oct. 10, '14: Prof. *F. P. Gay*, Experimental studies on methods of anti-typhoid immunization; Nov. 7: Prof. *A. S. Loevenhart*, Certain aspects of vital oxidation; Nov. 28: Prof. *L. B. Mendel*, Nutrition and growth; Dec. 12: Prof. *L. J. Henderson*, The excretion of acid in health and disease; Jan. 30: Prof. *Hans Zinsser*, The more recent developments in the study of anaphylactic phenomena; March 13: Prof. *E. P. Joslin*, Carbohydrate utilization in diabetes based upon studies of the respiration, urin and blood.

Patholog. Soc., Phila.; *Gross Lect.*, Oct. 22, '14: Prof. *F. P. Gay*, New uses of a specific skin test in certain of the infectious diseases.

Phila. Pediatric Soc.; *Packard Lect.*, Nov. 10, '14: Dr. *Rufus Cole*, Pneumococcus infection and immunity.

Royal Society, London; *Croonian Lect.*, June 11, '14: Prof. *E. B. Wilson*, The bearing of cytological research on heredity.

Univ. of Pittsburgh; Soc. for Biol. Research, *Mellon Lect.*, Feb. 27: Prof. *J. J. Abel*, Experimental and chemical studies of the blood and their bearing on medicine. (See page 250).

MISCELLANEOUS ITEMS. Amer. Chem. Soc., Phila. Sect., Feb. 18; Symposium on enzymes: Dr. *D. D. Van Slyke*, Methods of enzyme preparation; Prof. *A. E. Taylor*, Reversion of ferment action; Dr. *J. S. Hepburn*, Behavior of enzymes at low temperatures (see page 136); Dr. *J. A. Kolmer*, Ferments and immunity.—Univ. of Ill. Sect., Feb. 16: Dr. *J. H. Beal* (chair, Board of Trustees, U. S. Pharmacopeial Convention), The Pharmacopeia and National Formulary as legal standards.

British Assoc. Adv. Science, Sect. Physiol. (pres. address), Melbourne, Aug. 14: Prof. *B. Moore*, Value of research in development of national health.

Brown Univ., Mt. Holyoke Coll., and Wellesley Coll.: Dr. *Lillian Welsh*, American women in science.

Clark Univ., Founder's Day celebration address, Feb. 1: Dr. *R. S. Lillie*, The relation of universities to investigation.

Franklin Inst., State of Pa., Sect. of Physics and Chem., Oct. 1, '14: Dr. *Max v. Recklinghausen*, The ultra-violet rays and their application for the sterilization of water; Jan. 28: Dr. *Ulric Dahl-*

gren, The production of light by animals; Feb. 4: Dr. *Carl L. Alsborg*, Moisture in agricultural products.

N. Y. Acad. of Sciences, Mar. 22: Prof. *Raymond Dodge*, Incidence of the effect of moderate doses of alcohol on the nervous system.

N. Y. Bot. Garden, Oct. 10, '14: Dr. *J. H. Barnhart*, Carnivorous plants; Nov. 14: Dr. *H. H. Rusby*, Influence of radium on the production of field crops; April 3: Sources of quinin.

Physical Soc., London, Oct. 23: Sir *J. J. Thompson*, Ionisation.

Princeton Chem. Soc., Feb. 25: Prof. *Chas. Baskerville*, Physical chemistry and anesthesia.

Rockefeller Inst. The Cosmopolitan Club held its eighth meeting Dec. 14, '14, at Columbia Univ. in the morning, at the Rockefeller Inst. in the afternoon. (See pp. 259, 268.) At the afternoon session addresses and demonstrations were made by Dr. *D. D. Van Slyke* and Mr. *G. E. Cullen*, Determination of urea; Dr. *F. C. McLean*, Effect of disease on elimination of urea and chlorids as measured by Ambard's coefficient; Dr. *P. A. Levene*, Vitamins; Dr. *John Auer*, Experimental intradural injections of therapeutic sera.

Royal Institution, Jan. and Feb.: Prof. *C. S. Sherrington*, six lectures, The muscle in the service of the nerve.

Univ. of Rochester, Nov. 9: Dr. *P. F. Trowbridge*, Some problems of nutrition.

Washington Univ., Assoc. and Med. School; Dr. *Graham Lusk*—Dec. 30: The cost of ready-to-serve food; Dec. 31: The basis of animal calorimetry; Jan. 2: Metabolism in diabetes.

Prof. *J. C. Bose*, prof. of botany, Presidency Coll., Calcutta, India, has recently completed a world tour, through Europe and America, during which he delivered many lectures on the irritability of plants. Many of the lectures were given from biochem. standpoints. Prof. Bose's book on this general subject was referred to in the last issue of the *BIOCHEM. BULL.* (1914, iii, p. 540).

Grants. Austrian Acad. of Sciences—Prof. *v. Jauregg*, \$1,250: research on the etiology of goiter; Prof. *Honigschmidt*, \$600: work on the atomic weight of the radium elements; Prof. *Netolitsky*, \$375: study of the history of food stuffs.

Paris Acad. of Sciences, Bonaparte Fund; grants (francs) for 1914.—*Pierre Breteau*, 2000; use of palladium in analysis and in

organic chem.—*M. Chatton*, 2000; researches on the parasite *Peridinians*.—*Dr. Mauguin*, 2,000; liquid crystals.—*Dr. Chauvenet*, 2000; zirconium and its complex compounds.—*Prof. Lawvageau*, 2000; marine algae.

Prize. Paris Acad. of Science, 400 francs: *A. Lansenberg*, for his work on ammonia and urea—origin, methods of estimation.

Medals. PERKIN MEDAL: To *Dr. Edward Weston*, For distinguished achievement in the field of chem. engineering and metallurgy.—WILLARD GIBBS MEDAL: To *Dr. A. A. Noyes*, For devising an improved system of qual. analysis.

Journalistic. *Amer. Jour. of Surgery*. *Dr. Yandell Henderson* is a member of the staff which will publish, for the *Amer. Journ. of Surg.*, a quarterly supplement devoted to anesthesia. It has been adopted as the official organ of the Amer. Assoc. of Anesthetists and the Scottish Soc. of Anesthetists. The first issue of this supplement appeared in October.

Chemical Abstracts. *Dr. A. M. Patterson* resigned, last July, the editorship of *Chem. Abstr.* and was succeeded by *J. J. Miller*, one of the two assoc. ed. Since Jan., *E. J. Crane*, assoc. ed., has been the acting editor, aided by *Elmer Hockett*, assis. ed.

Of the total of 2,967 pages and 16,468 abstracts comprising *Chem. Abstr.*, for 1914, 706 pages and 4,692 abstracts were devoted to biol. chemistry.

Proc. Nat'l Acad. Sciences. The Nat'l Acad. of Sciences began, in Jan., the publication of monthly proceedings. (See page 275). The first paper in it of biochem. interest was published, in the Feb. issue, by *Drs. F. G. Benedict* and *Paul Roth*, on The basal caloric output of vegetarians as compared with that of non-vegetarians of like weight and height. Biochemistry is represented in the editorial board by *Prof. J. J. Abel*.

Funds and endowments. *Drs. W. J. and C. H. Mayo*, Rochester, Minn., established a \$1,000,000 foundation for med. research and presented the foundation, under certain restrictions, to the Board of Regents of the Univ. of Minn. Interest from the fund will be used for research at Rochester.

The Univ. of Oxford has received \$2,200 from friends of the late *Prof. Gotch* to perpetuate his memory and to encourage study of physiology. The income from the fund will be applied to the

establishment of a Gotch memorial prize to be awarded annually, after examination, to a student in the physiol. lab.; and to the creation and maintenance of a Gotch memorial library in the same lab.

Louis Moissan, son of the late Prof. Henri Moissan and assistant at the Ecole supérieure de Pharmacie, Paris (who died on the field of battle, August 10), has left to his school, in addition to the scientific books and apparatus of his father, 200,000 francs for the foundation of two prizes, one for chemistry (prix Moissan), and one for pharmacy (prix Lugan), in memory respectively of his father and mother, *néé* Lugan.

Through the generosity of Mr. R. B. Mellon an endowed lectureship has been presented to the Soc. for Biol. Research, Univ. of Pittsburgh, for an annual lecture on subjects of medical research. The lectures will be published in the form of monographs for distribution to libraries and investigators. The first lecture was given by Prof. John J. Abel (see p. 247).

New laboratories. The Henry S. Dennison memorial building for med. research, Univ. of Col., has been opened.

The new radium lab. of Manchester Infirmary has been formally opened by the Mayor of the city.

The new building of the Mellon Inst. of Indust. Research, Univ. of Pittsburgh, was dedicated on Feb. 26.

The Thomas W. Evans Museum and Dental Inst., Sch. of Dentistry, Univ. of Penn., was dedicated, Feb. 22-23.

The Chem. Section of the Iowa Agric. Exp. Sta'n has moved into the new \$250,000 Chem. Building of the Iowa State Coll., and occupies the entire southeast wing of the third floor.

Mr. A. Fleck, demonstr. at the Univ. of Glasgow, has been appointed physical chemist to the Glasgow Radium Commit., established to administer a large fund collected in that city for the purpose of acquiring and distributing radium for therapeutic purposes. A radiometric lab., under the auspices of the commit., has been fitted up at the univ.

Associations, societies, etc.: Officers-elect. ACAD. OF NATURAL SCI. Pres., *S. G. Dixon*; vice-p., *E. G. Conklin*, *John Cadwalader*; record. sec. and librarian, *E. J. Nolan*; cor. sec., *J. P. Moore*; treas., *George Vaux, Jr.*

AMER. ASSOC. ADV. SCIENCE. Pres., *W. W. Campbell*; perm.

sec., *L. O. Howard*.—Sect. A (Math.—Astron.), vice-p., *A. O. Leuschner*.—Sect. B (Physics), vice-p., *F. Slate*.—Sect. C (Chem.), vice-p., *Wm. McPherson*; member of council, *W. T. Taggart*; member of gen'l commit., *L. W. Jones*; member of sect. commit., *E. C. Franklin*.—Sect. D (Mech. Sci.—Eng.), vice-p., *B. J. Arnold*.—Sect. E (Geol.—Geogr.), vice-p., *C. S. Prosser*.—Sect. F. (Zool.), vice-p., *V. L. Kellog*.—Sect. G. (Bot.), vice-p., *W. A. Setchell*; member of council, *S. R. Jones*; member of gen'l commit., *W. L. Bray*; member of sect. commit. for 5 yr., *C. S. Gager*.—Sect. H (Anthrop.—Psychol.), vice-p., *G. M. Stratton*.—Sect. I (Soc.—Econ. Sci.), vice-p., *G. F. Kunz*.—Sect. K (Physiol.—Exp. Med.), vice-p., *F. P. Gay*; sec., *C.-E. A. Winslow*.—Sect. L (Educ.), vice-p., *E. P. Cubberley*.—Sect. M (Agric.), vice-p., *E. Davenport*; member of the gen'l commit., *A. C. True*; member of council, *W. A. Taylor*; member of sect. commit., *K. S. Butterfield*.

AMER. ASSOC. OF UNIV. PROF. Pres., *John Dewey*.

AMER. CHEM. SOC. Pres., *C. H. Herty*; directors (1915–1918), *Alexander Smith*, *E. G. Love*; councilors-at-large, *E. C. Franklin*, *F. K. Cameron*, *G. B. Frankforter*, *G. A. Hulett*.

AMER. HOME ECON. ASSOC. Pres., *Martha Van Rensselaer*; vice-p., *Abby L. Marlatt*, *Marion Talbot*, *B. R. Andrews*; sec., *Anna Barrows*; treas., *C. F. Langworthy*; members of the council, *Sarah L. Arnold*, *Isabel E. Lord*, *Josephine T. Berry*, *Catherine A. Muligan*, *Helen L. Johnson*.

AMER. PHYSIOL. SOC. See page 182.

AMER. SOC. OF BIOL. CHEM. See page 184.

AMER. SOC. FOR EXP. PATH. See page 188.

AMER. SOC. OF NATURALISTS. Pres., *F. R. Lillie*; vice-p., *R. A. Emerson*; sec., *Bradley M. Davis* (1914–16); treas., *J. Arthur Harris* (1915–17); addit. members of the ex. commit., *R. G. Harrison* (1914–15), *Raymond Pearl* (1914–16), *H. V. Wilson* (1915–17).

AMER. SOC. FOR PHARM. AND EXP. THERAPEUTICS. See page 186.

AMER. SOC. OF ZOOLOGISTS. Pres., *W. O. Lucy*; vice-p., *Wm. E. Ritter*; member at large of ex. commit., *D. H. Tennent*.

ASSOC. AMER. AGRIC. COLLEGES AND EXP. STA. Pres., *E. A. Bryan*; vice-p., *J. H. Worst*, *T. F. Hunt*, *C. D. Woods*, *P. H. Rolfs*,

C. A. Lovy; sec.-treas., *J. S. Hills*; bibliogr., *A. C. True*; exec. commit., *W. O. Thompson*, *H. J. Waters*, *Brown Ayres*, *W. H. Jordan*, *H. L. Russell*.

BIOL. SOC. OF WASH. Pres., *Paul Bartsch*; vice-p., *A. D. Hopkins*, *W. P. Hay*, *J. N. Rose*, *Mary J. Rathbun*; rec. sec., *M. M. Lyon, Jr.*; cor. sec., *W. S. McAtee*; treas., *W. W. Cooke*; members of council, *Hugh M. Smith*, *Vernon Bailey*, *Wm. Palmer*, *N. Hollister*, *J. W. Gidley*.

BOTAN. SOC. OF AMER. Pres., *J. M. Coulter*; vice-p., *R. A. Harper*; sec., *H. H. Bartlett*; treas., *Arthur Hollick*; councilor, *W. F. Ganong*.

FEDERATION OF AMER. SOCIETIES FOR EXP. BIOL. See page 179.

MASS. PUBLIC HEALTH ASSOC. Pres., *W. T. Sedgwick*.

SOC. OF AMER. BACTERIOLOGISTS. Pres., *D. H. Bergey*; vice-p., *John Weinzirl*; sec.-treas., *A. P. Hitchens*; council, *K. F. Kellerman*, *W. A. Stocking, Jr.*, *R. E. Buchanan*, *H. J. Conn*; deleg. to Amer. Assoc. Adv. Sci., *M. J. Rosenau*.

Miscellaneous items. FELLOWSHIPS. Wesley Memorial Hosp., Chicago, has established five fellowships to be given yearly to graduates in med. who aim to solve important problems applying to clinical med. and surg., or the specialties. The work will be done under a joint board selected from the staff of Wesley Hosp. and of the lab. dep's of the Northwest. Univ. Med. Sch.; the clinical work to be done in the hosp., and the lab. work in the lab. of the Med. Sch. The fellowships are open to any graduate in med. The recipient of the fellowship will be required to devote his entire time during the first year, at least, to investigation.

LUBRICANT FOR STOPCOCKS, ETC. An excellent lubricant for use with burette stopcocks, desiccators, etc., can be made by melting together equal parts of paraffin and vaselin. The paraffin gives body to the mixture, which is therefore superior to vaselin alone, especially in places where high temperatures prevail.

PROTEST AGAINST TAX ON DENTIFRICES. A commit. of N. Y. physicians and dentists has been formed to petition Congress to revoke the war tax on dentifrices, on the ground that such a tax is a severe blow to the work in progress, under the auspices of state and municipal public health agencies, in behalf of oral hygiene. The commit., of which Dr. H. L. Wheeler of the Coll. of Dental and

Oral Surg., is chair., includes Drs. W. S. Bainbridge, William Carr, H. Holbrook Curtis, Thomas Darlington, W. C. Dean, Francis Delafield, E. P. Fowler, Wm. J. Gies, S. S. Goldwater, V. H. Jackson, Ernest Lederle, O. V. Limerick, F. P. Miller and H. D. Pease.

FOR UNIFORMITY IN FOOD AND DRUGS LAWS. The Chamber of Commerce, U. S. A., a body composed of repr. from about 600 local boards of trade, chambers of commerce, and trade assoc. throughout the U. S., has taken up the study of uniform food and drug regulation. For this purpose a special commit. was appointed in July; its first meeting was held in Washington, Oct. 8. The commit. is composed of W. M. McCormick, Baltimore; A. J. Porter, Niagara Falls; John A. Green, Cleveland; B. L. Murray, and T. F. Whitmarsh, New York.

OFFICERS AND INVESTIGATORS, SPRAGUE INST. During the year 1914 the following persons have been connected with or have done work under the auspices of the Otho S. A. Sprague Memorial Inst.: *Director*: H. G. Wells.—*Members*: R. T. Woodyatt, Samuel Amberg, Lydia M. DeWitt, E. A. Graham, H. F. Helmholz, Maud Slye, H. J. Corper, E. J. Witzemann.—*Fellows*: Kaethe M. Dewey, W. B. McClure, F. W. Gaarde, W. D. Sansum, Grace Meigs, L. W. Sauer, A. B. Schwartz, W. H. O. Hoffmann, G. H. Coleman.—*Assistants*: Hope Sherman, Edith Farrar, Mary E. Maver.—*Voluntary associates, recipients of aid from or users of the facilities of the Inst.*: Frank Billings, P. S. Chancellor, Harriet F. Holmes, E. F. Hirsch, J. H. Lewis, G. T. Caldwell, H. L. Huber, E. W. Schwartz, J. J. Moore, E. R. Hayhurst, C. R. Spicer.

NEW PROCESSES: GASOLINE, BENZENE, TOLUENE. Sec'y of the Interior Lane has announced the discovery, by Dr. W. F. Rittman (chem. engineer in the Bureau of Mines, working at Columbia Univ.), of two chemical processes, one of which, it is claimed, will greatly increase the supply of gasoline, while the other may make the U. S. independent in regard to materials necessary for the dye industry and the manufacture of high explosives. Application has been made by Dr. Rittman, on behalf of the federal gov., for patents of these processes, in order to prevent monopoly in their use, the patents to be dedicated to the Amer. people.

PROHIBITION. I have been in the actuarial profession for over 20 years, and I have had the opportunity of studying not only the

published statistics, but many private investigations. I cannot recall a single class of men or women using alcohol freely but not immoderately at the date of application for insurance, or who had used it in excess formerly and were now temperate, that did not have a higher mortality than the normal. While not a total abstainer, I am convinced that it would be immeasurably better for this, or any other country, to have the production and sale of alcoholic liquors abolished if it were practicable. The advantages claimed for alcohol are a small offset, in my judgment, to the evils which proceed from its use and its abuse. *Arthur Hunter* (Med. Review of Reviews, 1915, xxi, p. 25).

II. WAR NOTES

Necrology.—*Max Brandt*, assis., Botan. Museum, Berlin-Dahlem.—*Philip Beck*, head of the Austrian Army Med. Staff.—*Hans Halle*, assis. plant physiol., Univ. of Munich.—*Oswald Loeb*, docent for pharmacol., Univ. of Göttingen.—*Franz Marshall*, director of the exper. lab., Agric. Inst., Univ. of Halle.—*Wilhelm Schneider*, assis., Agric. Inst., Giessen.—*R. Stumpf*, docent and first assis., Pathol. Inst., Univ. of Breslau.—*Alfred Tournier*, formerly prof. of viticulture, Univ. of Cal., later connected with the U. S. Dep't of Agric.

Awards of the Iron Cross. To *Walther Nernst*, prof. of physics, Univ. of Berlin, who, since the death of his son at the front, has joined the automobile corps.—To Dr. *Karl Thomas*, of Prof. Rubner's lab., Berlin.

University items. GENERAL. Two thirds of the number of students at Oxford and Cambridge Univ's have enlisted in the British army. Trinity Coll. has been converted into a military hosp.

Prof. R. du Bois-Raymond, writing in the *Berliner Tageblatt*, says that from Berlin Univ. 236 lecturers, nearly half the total number, are in the army, either voluntarily or in obedience to law. The med. faculty furnished 133 men, presumably for the med. service of the army.

Of the 2,069 German students at Tübingen last semester, 1,500 are at the front, and several hundred are in the med. service.

The Harvard Univ. corporation has set aside \$100,000 to pay

professors who have been driven from Belgium and may give courses at Harvard Univ. next year.

PERSONAL. The Imperial Soc. of Naturalists, Moscow, has removed the names of Prof's Haeckel and Ostwald from the list of members because they signed the address "To Civilized Nations."

In answer to the manifesto of the "German intellectuals," which is considered as unifying German culture and German militarism, La Société Nationale d'Acclimatisation de France has removed from its list of members all Germans and Austrians.

Among the German scientific men who have affixed their names to a manifesto renouncing the honors conferred upon them by English univ's and other learned institutions are Prof's Ehrlich, v. Behring, Haeckel, Weismann, Wundt, Lenard and Roentgen.

Prof's Waldeyer, Orth and others have added their protest to that of Prof's Foerster and Verworn against the action of Prof's Ehrlich, v. Behring, Roentgen and others, in melting down the medals and renouncing the honors conferred upon them by various scientific bodies in Great Britain.

HONORARY DEGREE. The Univ. of Königsberg has bestowed an honorary M.D. upon Gen. v. Hindenburg "for making the Russians take their medicine."

Chemical items. **ANILIN DYES.** A special commit. of N. Y. chemists, appointed to investigate and report upon conditions and needs involved in the enlargement of the coal-tar dye industry in the U. S., have published their report in the *Jour. Ind. and Eng. Chem.* (Dec., 1914).

DRUGS. The Austrian gov. has prohibited the exportation of hosp. supplies, lab. animals, vaccins, and various drugs and chemicals, including phenol, mercury, iodine, bismuth, strychnin, morphin, etc.

RADIUM. The European war has, for the present at least, totally closed the European market to Amer. radium ores. As is well known, the uranium ores of Colorado and Utah are sold exclusively for their radium content, so little use being known for uranium that the ores can not be sold for their content of that element. The closure of the European market leaves but one known buyer, so that while the war lasts and probably for some time after-

wards, the market will be restricted and without the benefit of competition.

THYMOL. Hitherto thymol has been almost entirely manufactured in Germany, although the ajowan seeds, which are almost the sole source of the oil from which thymol is produced, are grown on a large scale only in India. The cutting off of the supplies of thymol from Germany has increased the price eightfold, and it is even now (Jan. 15) \$5 a pound, as against \$1.25 before the war. As the manufacturing process is quite simple, preparations are now being made to produce the drug in England. A British possession is able to provide a substitute for thymol, *carvacrol*, obtained from oils derived from a variety of plants, but particularly from the origanum of Cyprus.

SODIUM VERSUS POTASSIUM SALTS. The probable shortage of potassium salts, due to the war, suggests that sodium salts may in most cases be substituted without disadvantage. In general, potassium salts have no marked superiority over the corresponding sodium salts. While the potassium compounds are said to be more active and to possess a more diuretic effect, the sodium salts are less depressing to the heart and in some instances less disagreeable to the taste. Sodium iodid, sodium bromid, sodium acetate, sodium citrate, etc., are just as effective as the corresponding potassium salts.

Miscellaneous items. In consequence of the war, the publication of the British Pharmacopoeia for 1914 has been postponed.

The weekly French scientific journal, *La Nature*, which suspended publication in August, began again on Dec. 12.

The family of Emil du Bois Reymond has donated the Helmholtz gold medal to the relief fund, with the statement that this medal, representing the highest appreciation in his own land of the scientific achievements of du Bois Reymond, is honored more by devoting it to the service of his country than by preserving it.

The *Münch. med. Wochenschr.* has proposed that a select committee prepare a list of German equivalents for the names of diseases which have been taken from the Russian, French and English languages; if no satisfactory German names can be found, Latin or Greek to be substituted.

The Rockefeller Inst. for Med. Research has appropriated \$20,000 to be used, under the direction of the Inst., for the furtherance

of med. research under war conditions, and is equipping Dr. Carrel's new hosp. in France with apparatus for research along pathol., bacteriol., surg. and chem. lines. Dr. H. D. Dakin is associated with Dr. Carrel in this work.

Dr. C. D. Walcott, sec'y of the Smithsonian Institution, has been informed that the Stazione Zoologica at Naples is in a somewhat serious condition financially, owing to the withdrawal of German support. The Smithsonian Institution maintains a table at the station, which is all it can do under existing conditions. Dr. Walcott's informant suggests that if our univ's would take up some of the vacated tables, it would not only assist the station, but would eventually result in closer cooperation between our scientific men and those of Europe.

III. COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

I. General notes

Appointments. Columbia Univ.: Dr. *B. S. Oppenheimer*, assis. prof. of clin. med., Montefiore Home (promotion).—Dr. *A. D. Dryfoos*, clin. assis., and Dr. *H. J. Wiener*, assis., in med., Vanderbilt Clinic.—Dr. *O. C. Pickhardt*, assis. in anatomy.—*Helene M. Pope*, assis. in dietetics (Teach. Coll.); *Elizabeth G. Van Horne* (Rochester A. and M. Inst.), Caroline Scholar, Sch. of Practical Arts (Teach. Coll.).

Conn. Coll. for Women (New London): Dr. *R. C. Osburn* (Columbia Univ.), assis. prof. of zool.

Cornell Univ.: *Mabel C. Little* (N. Y. Polyclin. Med. Sch.), director of the dining rooms and instr. in institutional administration.

General Bakelite Co., N. Y.: *M. L. Hamlin* (Harriman Research Lab.), chemist.

Home for Incurables (Newington, Conn.): *R. A. Yergason* (Trinity Coll.), pathologist and bacteriologist, and attending surgeon.

Marine Biol. Lab. (Woods Hole, Mass.): Dr. *Chas. Packard*, instr. in zoology (summer, 1915).

N. Y. Botan. Garden: *Helene M. Boas* (Col. Univ., Barnard Coll.), assis.

N. Y. City Board of Health: Dr. *C. F. Bolduan*, director of public health education (promotion); *L. J. Hirshleifer* (N. Y. State Food Inspec. Lab., N. Y. City), chemist.

Penn. State College: *J. P. Kelly*, instr. in botany.

Royal Columbian Hosp. (New Westminster, B. C., Can.): *Blanche R. Harris* (State Normal School, Truro, N. S., Can.), dietitian.

U. S. Dep't of Agric., Bureau of Chem.: Dr. *L. E. Wise* (Univ. of Mo.), biochemist (pharmacol. lab.); Dr. *H. E. Woodward* (U. S. Food and Drug Inspec. Lab., Phila.), transferred to the Bureau of Chem. (Wash.).

Univ. of Ala., Grad. Sch. of Med. (Birmingham Med. Coll.), Sch. of Pharm.: Dr. *A. R. Bliss*, dean (promotion).

Univ. of Ill., Med. Coll. (Chicago): Dr. *C. S. Smith* (U. S. Dep't of Agric., Bureau of Chem.), instr. in physiol. chem.

Univ. of Ind.: Dr. *Mildred A. Hoge*, instr. in zool.

Vacuum Oil Works (Rochester, N. Y.): Dr. *D. F. Renshaw* (Rochester High Sch.), chemist.

Yale Univ.: Dr. *L. L. Woodruff*, prof. of biol. (promotion).

Associations and societies. OFFICERS-ELECT. Dr. *C. L. Alsberg*: pres't Wash. Sect., Amer. Chem. Soc.; vice-p. (reelected), Amer. Soc. Biol. Chem.

Dr. *George D. Beal*: sec'y Amer. Chem. Soc. (Univ. of Ill. Sect.).

Prof. *S. R. Benedict*: member, Nominating Commit., Amer. Soc. Biol. Chem.

Prof. *Josephine T. Berry*: member, Council, Amer. Home Econ. Assoc.

Dr. *Leo Buerger*: chair., Sect. of genito-urinary diseases, N. Y. Acad. of Med.

Dr. *Norman E. Ditman*: member, Board of Trustees, chairman, dep't of hygiene and sanitation, America's 2d Expos. of Safety and Sanitation, Safety and San. Conference, Grand Central Palace, N. Y. (under the auspices of the Amer. Museum of Safety), Dec. 14-19, '14.

Dr. *Nellis B. Foster*: sec'y, Sect. on Med., N. Y. Acad. of Med.

Dr. *C. Stuart Gager*: councillor, N. Y. Acad. of Sciences; member, Sect. commit., Sect. G (Botany), Amer. Assoc. Adv. Science.

Prof. *Mary E. Gearing*: pres't, Home Econ. Assoc. of Texas.

Dr. *R. F. Hare*: Grand Master, Masons of New Mexico.

Dr. *T. Stuart Hart*: chair., Sect. of Med., N. Y. Acad. of Med.

Prof. *P. B. Hawk*: member, Nom. Commit., Amer. Soc. Biol. Chem.

Dr. *J. S. Hepburn*: member, Exec. Commit., reappointed member of program commit., Phila. Sect., Amer. Chem. Soc.

Louise McDanell: acting sec'y, meeting of the Household Econ. Sect., Nat'l Educ. Assoc., St. Paul, July 9.

Dr. *S. J. Meltzer*: member, Membership Commit., Amer. Soc. f. Pharm. and Exp. Therap.

Prof. *Lafayette B. Mendel*: member, Council, Amer. Soc. Biol. Chem.

Prof. *Raymond C. Osburn*: vice-p., N. Y. Acad. of Sciences.

Prof. *A. N. Richards*: member, Nom. Commit., Amer. Soc. Biol. Chem.

Prof. *H. von W. Schulte*: member, Exec. Commit., Amer. Assoc. of Anatomists.

Prof. *Alexander Smith*: director, Amer. Chem. Soc.

Prof. *Mary E. Sweeny*: pres't, Home Econ. Assoc. of Kentucky.

MEMBERS-ELECT. Dr. *A. T. Cameron*: Amer. Physiol. Soc.; Amer. Soc. Biol. Chem.

Mary C. de Garmo: Amer. Chem. Soc.

Dr. *E. N. Harvey*: Amer. Physiol. Soc.

Dr. *Sergius Morgulis*: Amer. Physiol. Soc.

Dr. *Max Morse*: Amer. Soc. Biol. Chem.

Prof. *H. von W. Schulte*: fellow, N. Y. Acad. of Sciences.

Addresses and lectures. Allegheny Co. Med. Soc., Oct. 20: Dr. *Jacob Rosenbloom*, Hemolytic jaundice.

Amer. Chem. Soc., Ill. Sect., Dec. '14: Prof. *Isabel Bevier*, Household chemistry.—St. Louis Sect., Mar.: Dr. *Sidney Born*, Acetolysis of carbohydrates.—Minn. Sect., Feb.: Dr. *R. A. Gortner*, Animal pigments.—Phila. Sect., Feb.: Dr. *J. S. Hepburn*, Enzymes (p. 136).—Wis. Sect., Dec.: Dr. *Max Morse*, The effect of certain organic iodine compounds on growth and development.

Coll. of Phys. (Phila.), Sect. on Med., Dec. 14, '14: Dr. *Alfred E. Cohn*, Clinical and electrocardiographic studies on the action of digitalis.

Col. Univ., Sigma Xi, Mar. 24: Mr. *H. J. Muller*, Some recent work in heredity; Dr. *Chas. Packard*, The effect of radium on living matter.—The Cosmopolitan Clinical Club held its eighth meeting, Dec. 14, '14, at Columbia Univ. (Crocker Lab., and Coll. of Phys. and Surg.) and the Rockefeller Inst. Dr. *Wm. H. Woglom* discussed some phases of the cancer problem; Prof. *Hans Zinsser* made a Demonstration of work in the Columbia dep't of bacteriology (see page 268).

Harvard Univ., Boylston Chem. Club, Feb. 26: Prof. *Alexander Smith*, The forms of sulfur and their relations.

Johns Hopkins Hosp. Med. Soc., Mar. 1: Dr. *Lafayette B. Mendel*, Nutrition and growth.

N. Y. Acad. of Med., Harvey Lect.—Nov. 28, '14: Prof. *Lafayette B. Mendel*, Nutrition and growth; Prof. *Hans Zinsser*, The more recent developments in the study of anaphylactic phenomena.

N. Y. Bot. Gard.: Dr. *C. Stuart Gager*, Sept. 5, '14, Life history of a tree.—Dr. *F. J. Seaver*, Oct. 3, '14, Economic importance of fungi; May 29, Destructive insects.

N. Y. Univ. and Bell. Hosp. Med. Coll., Herter Lect., Dec. 10, 11, 14, 15, '14: Prof. *Lafayette B. Mendel*, Aspects of the physiology and pathology of growth.

Penn. State Med. Soc., Sept. 24, '14: Dr. *Jacob Rosenbloom*, A form of diabetic coma without any relation to acetone substances.

Round Table Club, Pittsburgh, Nov. 10, '14: Dr. *Jacob Rosenbloom*, Research and medicine.

Univ. of Penn., 9th Rush Society lect., Jan. 22: Prof. *Lafayette B. Mendel*: Nutrition and growth.

West Va. State Med. Soc., Nov. 2, '14: Dr. *Jacob Rosenbloom*, Infection and immunity.

Miscellaneous items. At its Commencement last June, Jefferson Med. Coll. conferred higher degrees in course for the first time. All the candidates were from the Dep't of Physiol. Chem., of which Prof. *P. B. Hawk* is the head. *Olaf Bergeim*, Ph.G., M.S., received the degree of Ph.D.; thesis, A study of calcium metabolism in certain pathological conditions. *Clarence A. Smith* and *James T. Leary* received the degree of M.S.

New Hampshire State Coll. began the organization of a department of Home Economics in May 1913. The work was placed under the direction of Miss *Helen B. Thompson* and classes were opened in Sept. A splendid equipment was installed during the summer. In response to the announcement of the course, the enrollment of women increased to double the number for the preceding year. Sixty-three women have been registered, most of them taking work in the dep't and many of them entering the full course.

The first number of the *Proc. Nat'l Acad. Sciences* (see p. 275) contains a paper (p. 44) by Dr. *Jacques Loeb* and *Hardolph Wassteneys*, On the identity of heliotropism in animals and plants.

Prof. *Wm. H. Welker* served on the catalog commit. of the Coll. of Med., Univ. of Ill., and is also sec'y *pro tem* to the Jr. Fac-

ulty for the present year. He is making an extensive study of the teaching faculties of the better med. schools.

Prof. Hans Zinsser is a member of the Sanitary Commis. organized by the Amer. Red Cross and the Rockefeller foundation, which recently went to Serbia to aid in the control of typhus fever, now epidemic there.

2. Proceedings of the Association

EDGAR G. MILLER, JR., Secretary

Eighteenth-twentieth meetings. Abstracts of the papers comprising the scientific proc. of the 18th, 19th and 20th scientific meetings of the Assoc. are given at pages 193, 210, and 233, resp.

First quarterly informal dinner. Immediately after the adjournment of the 20th meeting (Feb. 5), the members in attendance proceeded to the Hofbrau Haus, 29th St. and Broad., where they greatly enjoyed an informal dinner. Dr. Gies, the guest of honor, spoke informally and intimately of the past and present of the Biochem. Dep't; and suggested ways and means for the steady improvement of the work in biochemistry, and for a cumulative increase in the usefulness of the Biochem. Dep't and the Assoc. The great success of this informal dinner from every standpoint led to a subsequent decision to make such events a feature of the quarterly meetings of the Assoc. (See page 266).

Fourth annual dinner (21st meeting). All arrangements had been completed for the fourth annual dinner of the Biochem. Assoc. on Dec. 11, '14, with Dr. S. J. Meltzer the guest of honor, when a serious attack of pneumonia (Dec. 7) made Dr. Meltzer's attendance impossible. The dinner was accordingly postponed to Mar. 26, when it was held with Dr. Meltzer again in good health. The "grand ball room" at Reisenweber's (8th Ave. and 58th St.) was completely filled by the 200 members and guests who were present.

Among the out-of-town members and guests in attendance were Drs. John S. Adriance, F. G. Benedict, W. B. Cannon, C. B. Davenport, Casimir Funk, E. N. Harvey, Yandell Henderson, J. S. Hepburn, Wm. H. Howell, F. H. McCrudden, John Marshall, L. B. Mendel, Leverett Mears, W. A. Noyes, G. Delgado Palacios.

The president of the Assoc., Dr. A. J. Goldfarb, was the toastmaster. The speakers who preceded Dr. Meltzer on the informal program were Drs. W. H. Howell, Jacques Loeb, L. B. Mendel, F. H. McCrudden and Chas. Baskerville. Drs. E. G. Conklin, Simon Flexner and Graham Lusk had accepted invitations to address the Assoc. informally, but illness of themselves or near relatives made their attendance impossible.

Just before Dr. Meltzer gave the address of the evening, Dr. Benjamin Horowitz, seconded by Dr. E. G. Miller, Jr., nominated Dr. Meltzer for honorary membership in the Biochem. Assoc. In his nominating speech Dr. Horowitz said that the spirit in which he made the nomination was indicated by the following quotation from the official letter in which the Assoc. conveyed its invitation to Dr. Meltzer to be the guest of honor at this dinner:

. . . You have had a far greater influence on us than you have ever imagined. Your splendid fidelity to your scientific ideals during the period when research facilities were lacking, and discouragement met you everywhere, has been an inspiring and an ennobling example. Your loftiness of purpose and your consecration to the pursuit of truth, as well as your industry, perseverance, thoroughness, imagination, fertility and constructive achievements, have been not only a revelation of idealism, and of power and service at their best, but also have been productive of practical results where you least expected to find them. They have had pronounced effects generally and they will continue to induce them. They have induced in us, for example, the earnest desire to emulate your personal and professional qualities; to achieve, if possible, as worthily, at least in spirit; and to hand on 'down the line' cumulative influences for good.

. . . We often think that as our years increase we are like the mountain climber who, as he mounts, notices less and less of the details in the view about him and regards more and more the outstanding big things. The spiritual are the big things; and what can be larger than the realization that one's personality, example, fidelity, achievement and idealism have not only won from a younger generation its admiration, esteem and gratitude, but have inspired it to its loftiest purposes for its own day and to its worthiest aspirations for the generations to succeed it. If such a realization can give you the satisfaction that we think it must, be assured that your splendid influence on the Columbia biochemical family will be an *abiding* influence

in our 'family' councils and always a cherished memory in our hearts individually.

We tender you this special invitation with our affectionate greetings and cordial good wishes.

Dr. Meltzer was then unanimously elected to honorary membership. After his introduction by the toastmaster, and informal allusions to some of the remarks of his predecessors on the program, Dr. Meltzer delivered a notable address on The deplorable contrast between *intranational* and *international* ethics, and the mission of medical science and medical men.¹

At the conclusion of Dr. Meltzer's address, Dr. Gies spoke informally in cordial appreciation of the address and in hearty support of the proposal that "medical men of various shades and groupings ought to establish a Medical Brotherhood for the Purpose of Upholding and Accelerating the Progress of International Morality." Dr. Gies said that if, in the estimation of Dr. Meltzer, the Biochem. Assoc. might serve in any way as an enzyme to "accelerate the progress" to which Dr. Meltzer alluded, he (Dr. Gies) would urge the Assoc., at its quarterly meeting on Apr. 9 (see page 267), to take official action in support of Dr. Meltzer's proposal. Dr. Meltzer thereupon moved, seconded by Dr. Gies, that all present at the dinner be invited to express themselves, by a rising vote, in favor of or in opposition to the plan suggested by Dr. Meltzer. The vote was unanimously in the affirmative.

Prior to adjournment Dr. Gies announced that Dr. Casimir Funk would be the guest of the Assoc. at its second quarterly dinner on Apr. 9 (see page 266), and that Dr. Funk would then address the Assoc. on the subject of Vitamins.

The names of those present, and the table groupings (31), are indicated below:

¹ This address was published, in extenso, in *Science*, xli, pp. 515-523 (April 9).

Speakers' Table

*Charles Baskerville	*Casimir Funk	*Francis H. McCrudden
*Francis G. Benedict	A. J. Goldfarb	*John Marshall
*Franz Boas	(<i>Toastmaster</i>)	S. J. Meltzer
*W. B. Cannon	*Yandell Henderson	Lafayette B. Mendel
*†J. McKen Cattell	*L. Emmett Holt	*W. A. Noyes
*Warren Coleman	*W. H. Howell	*G. Delgado Palacios
*†E. G. Conklin	*†H. C. Jackson	*George B. Wallace
*Charles B. Davenport	†S. W. Lambert	*Edmund B. Wilson
*James Ewing	Jacques Loeb	*C.-E. A. Winslow
*†Simon Flexner	*†Graham Lusk	
John S. Adriance		*†S. P. Beebe
*Mrs. Wm. J. Gies		*William Carr
*Mrs. Paul E. Howe		*Wm. B. Dunning
*Leverett Mears		*Henry W. Gillett
*Mrs. S. J. Meltzer		*Warfield T. Longcope
*Mrs. Victor Meltzer		*Hideyo Noguchi
*Victor Meltzer		*Herbert L. Wheeler
*Mrs. T. H. Morgan		*Carl J. Wiggers
* Gary N. Calkins		*John Auer
*†C. C. Curtis		*W. A. Bastedo
C. Stuart Gager		*Thos. S. Githens
E. Newton Harvey		*Robert A. Hatcher
*P. A. Levene		*D. S. D. Jessup
*T. H. Morgan		†C. C. Lieb
†Alexander Smith		*†Fenton B. Turck
*R. S. Woodworth		
*James P. Atkinson		*Jerome Alexander
*Edwin J. Banzhaf		*L. H. Baekeland
*William P. Healy		*Marston T. Bogert
*Wm. G. Lyle		*†C. F. Chandler
*Arthur H. Merritt		*†C. A. Doremus
*W. D. Tracy		*Tsaac F. Harris
*L. M. Waugh		*Herbert D. Pease
		*E. E. Smith
*Henry S. Dunning		Arthur F. Chace
*Henry C. Ferris		*†W. L. Estabrook
*Cyrus W. Field		*Morris S. Fine
*†F. B. La Forge		Michael Heidelberg
*John H. Larkin		*Philip A. Kober
*I. Levin		Gustave M. Meyer
*Charles C. Linton		*Victor C. Myers
*Charles Norris		Matthew Steel
*Richard Weil		

* Guest.

† Detained or obliged to leave before the conclusion of the dinner.

Alfred F. Hess
 *J. G. Hopkins
 *W. A. Jacobs
 *N. W. Janney
 *H. C. Sherman
 *D. D. Van Slyke

*E. A. Aronson
 Samuel Gitlow
 *C. Goldmark
 †Donald Gordon
 Abraham Gross
 †Otto C. Pickhardt
 †O. M. Schloss
 Wm. Weinberger

Lucy F. Cooper
 Isabel Clegg
 †Robert Grosvenor
 †Genevieve Howell
 John Sherburne, 2d
 Harold C. Tooker

Ernst P. Boas
 Helene A. Boas
 Sergius Morgulis
 A. Mutscheller
 William A. Perlzweig
 Anna B. Yates

Sol Biloon
 *Frederick E. Breithut
 Louis J. Curtman
 B. G. Feinberg
 *William L. Prager
 *Michael Ringer
 †G. Scatchard
 †H. Wasteneys

*N. R. Blatherwick
 *K. G. Falk
 I. Greenwald
 *J. T. W. Marshall

Hattie L. Heft
 *Alfred H. Rahe
 Jessie M. Rahe
 *Eleanor Van Alstyne

* Guest.

† Detained or obliged to leave before the conclusion of the dinner.

Arthur M. Buswell
 Katherine R. Coleman
 *Israel S. Kleiner
 Grace MacLeod
 Anton R. Rose
 *Mary D. S. Rose

Blanche Cooper
 Irene S. Dougherty
 †Ethel Epstein
 Beatrix H. Gross
 Tula L. Harkey
 Lottie M. Hull
 Almeda Perry
 Grace C. Robinson

Harvey B. Clough
 Hazel Donham
 Helen Gavin
 Fred W. Hartwell
 Mary G. McCormick
 Helene M. Pope

Robert Bersohn
 Israel J. Kligler
 Louis Pine
 Geo. J. Rosenthal
 Alfred V. Salomon
 Charles Weisman

O. C. Bowes
 C. H. Farr
 Joseph S. Hepburn
 Benjamin Horowitz
 Paul E. Howe
 C. M. Jordan
 S. Kubushiro
 E. G. Miller, Jr.

*Elizabeth V. Gaines
 Emily C. Seaman
 *Caroline E. Stackpole
 Jennie A. Walker

Louise C. Ball
 *James P. Haney
 Victor E. Levine
 Jeannette C. Mullikin

*Alma J. Finlayson
 Lucy H. Gillett
 Helen McClure
 *(Miss) E. A. Winslow

Bertha N. Baldwin
 Emma L. Kemp
 Leila J. Wadsworth
 *Chas. W. Wadsworth

Gladys Beckett
 Mabel Cain
 *†Sarah S. Graves
 Cornelia Luce

†H. L. Fisher
 Benjamin S. Kline
 *Arthur R. Mandel
 *J. M. Nelson

* Guest.

† Detained or obliged to leave before the conclusion of the dinner.

Jenoise Brown
 Helen Coombs
 *Alma Oswald
 Grace Sheets

Bessie Chamberlayne
 Lilla A. Harkins
 Jessie Johnston
 Mrs. J. S. Schapiro

*C. H. Allan
 G. E. Cullen
 A. D. Emmett
 *C. J. West

D. B. Armstrong
 *J. J. Connellan
 J. G. Dwyer
 Wm. J. Gies

DINNER COMMITTEE: Drs. Emily C. Seaman (chair.), Edgar G. Miller, Jr. (sec'y) and Paul E. Howe (treas.).

Twenty second meeting.² The third quarterly meeting of the Assoc. (1914-'15) was held in the Biochem. Seminar Room as usual, at 5 p. m., on April 9. Abstracts of the papers comprising the scientific proc. of this preliminary session will be published in the June issue of the *BIOCHEM. BULL.*

At 6.30, after the conclusion of the preliminary session, about 40 members adjourned to the Med. Sch. Library, where an informal dinner was served, with Drs. Casimir Funk and A. B. Macallum, Jr., the guests of the Assoc. and the pres't of the Assoc., Dr. Goldfarb, "at the head of the table." Informal conferences continued very enjoyably until 8 o'clock, when about 100 additional members and guests, who had assembled in a near-by lecture room, were admitted to the dining room to hear Dr. Funk's address on Vitamins, which was interesting and instructive in high degree. Informal remarks on the address were then made by Pres't Goldfarb and Drs. A. B. Macallum, Jr., A. F. Hess and S. J. Meltzer. Dr. W. H. Eddy voiced both the appreciation with which the Assoc. had listened to

² Unavoidable delay in the publication of this number makes it possible for us to include reference to this meeting.

and profited from Dr. Funk's address, and the pleasure that the presence of Drs. Funk and Macallum at this meeting had given to the members.

The meeting was concluded with an open executive session at 9.15, which was attended by practically all who heard Dr. Funk. At the invitation of the chair Dr. Meltzer restated and explained informally his suggestion at the fourth annual dinner regarding the formation of a "brotherhood for the acceleration of progress in the development of international morality" (page 263). Dr. Gies then took the floor in support of Dr. Meltzer's idea. He suggested that the day on which this meeting was held was peculiarly appropriate for the formal inauguration of a movement that might accelerate the progress of international morality and increase the security of international peace: *April 9*, the 50th anniversary of the noblest episode in American history, when Grant and Lee shook hands at Appomattox, and the golden era of interstate peace began in this country! Dr. Gies moved, seconded simultaneously by several, that a committee be appointed, with the Pres't of the Assoc. a member, to further Dr. Meltzer's project; and that the committee be instructed to endeavor to quicken the interest of Amer. med. men in the plan until the efforts of the Assoc. in this regard become an integral part of a more general movement by the med. men of this and other countries.

This motion was adopted by unanimous vote. The chair stated that the personnel of this committee would be publicly announced at an early date. The meeting thereupon adjourned.

The next meeting of the Assoc. will be the concluding quarterly session for 1914-'15, on May 28, at 5 p. m.; it will be followed by an informal dinner at 6.30 p. m.

3. Columbia Biochemical Department

Appointments. FROM THE STAFF. Beth Israel Hosp. (N. Y.): Dr. *E. G. Miller, Jr.*, biol. chem., to succeed Dr. *Max Kahn* until July 1. (Dr. Miller retains his associateship in biol. chem.)

Nat'l Pathol. Lab. (N. Y. City): *Tula L. Harkey*, head of the dept of chemistry and bacteriology.

N. Y. Manual Training High Sch.: *Robert Bersohn*, assis. teacher of chem.

U. S. Bureau of Fisheries: Dr. *Sergius Morgulis*, in charge of in-

vestigations of the metabolism of fishes, conducted in the N. Y. Aquarium and Col. Univ. Biochem. Lab.

West. Penn. Hosp. (Pittsburgh): Dr. *Max Kahn*, biol. chemist, to succeed Dr. *Jacob Rosenbloom*, resigned.

FROM THE BODY OF ADVANCED STUDENTS. U. S. Bureau of Mines (Wash.): *Gustav Egloff*, assis. to Dr. W. F. Rittman, discoverer of a new process for the production of benzene and toluene, and of increased yields of gasoline from crude petroleum. (See page 253.)

Univ. of Calif.: *Agnes F. Morgan*, assis. prof. of nutrition.

Appointments to the staff. *Katherine R. Coleman* (Turck Research Lab.), assis. (Teach. Coll.); research assis. ("P. and S.").—*Helen C. Coombs*, assis. (Teach. Coll.).

Associations and societies. OFFICERS OF THE DEP'T. Amer. Chem. Soc.: *Robert Bersohn*, *Tula L. Harkey* and Dr. *E. G. Miller, Jr.*, members.

N. Y. Acad. of Sciences: Dr. *V. E. Levine*, member.

ADVANCED STUDENTS. Amer. Chem. Soc.: *O. C. Bowes* and *Alexander Lowy*, members.

Addresses and reports. Amer. Chem. Soc., Phila. Sect., Dec. 17, '14: Dr. *Wm. J. Gies*, Chemical investigation of the cause and prevention of dental caries.

First Dist. Dental Soc. (N. Y.), Nov. 2, '14: Dr. *Wm. J. Gies*, Annual report on research in dental chemistry—(1) Further study of the effects of acid on natural extracted teeth (with Drs. *A. P. Lothrop*, *H. W. Gillett*, *C. C. Linton*, *A. H. Merritt* and *H. L. Wheeler*); (2) Distribution of trypan blue, after injection into living albino rats (with Dr. *E. G. Miller, Jr.*).

Nu Sigma Nu Alumni Assoc., Yale Club, N. Y., Nov. 24, '14: Dr. *Wm. J. Gies*, Dental caries.

The Cosmopolitan Clinical Club held its eighth meeting, Dec. 14, '14, at Columbia Univ. [Crocker Lab. (9.30–10.45 a. m.) and "P and S" (11–12.30 p. m.)] and at the Rockefeller Inst. (2.30–5.00 p. m.). The first meeting at "P and S" was held in this lab. (11–11.30 a. m.), and was addressed by Dr. *Gies*, by invitation, on Dental caries. (See page 259.)

Miscellaneous items. Dr. *E. G. Miller, Jr.*, has been cooperating (since Jan.) with Drs. *Gies* and *Howe*, in the editorial management of the biochem. dep't of *Chemical Abstracts*.

Dr. *Emily C. Seaman* has been appointed, by the Commis. on Prison Reform, to investigate the dietary efficiency and conditions of the State Prison of N. Y.; to report on the result of the investigation; and to cooperate with the Commis. in bringing about desirable changes. Dr. Seaman has also been made a member of the Commit. on Social Hygiene of the Nat'l Commit. on Prisons and Prison Labor.

Misses Lucy F. Cooper and Leila J. Wadsworth have been appointed Practical Arts Scholars (Teach. Coll.).

Dr. *Gies* has been elected a member of the N. Y. Sabbath Commit.; an honorary pres't of the Panama-Pacific Dental Congr., San Francisco, Aug. 30-Sept. 9; member of the Honorary Council of the 14th Internat'l Lord's Day Congr., Oakland, Cal., July 27-Aug. 1; member of the School Lunch Commit., N. Y. Assoc. for Improving the Condition of the Poor; chair. of the sub-commit. of the Advisory Council, N. Y. Board of Health, to deal with the problem of the shipment of fat into N. Y. City; member of a commit. of N. Y. physicians, surgeons and dentists which petitioned Congress to revoke the war tax on dentifrices (p. 252).

EDITORIALS

WILLIAM J. GIES

In our last preceding issue we apologized to our subscribers for the unavoidable delay which had characterized the issuance of the successive numbers of the **BIOCHEMICAL BULLETIN**, from the beginning of its career. When that apology was written we intended to begin Vol. IV with the January issue. Further difficulties connected with the printing of this number, and the ensuing delay, induced us, on Mar. 15, to send to the subscribers the following announcement:

The number of the **BIOCHEMICAL BULLETIN** now in press is the largest ever issued. It greatly exceeded our expectations in difficulties attending its printing and its size, and unavoidable delay in its publication has ensued. Instead of making this number the January issue, as we intended to do, it will be credited to March and the four parts of Volume IV will continue, as previously planned, to coincide with the calendar year 1915. This adjustment of our quarterly issues to the conventional Mar.-June-Sep.-Dec. sequence will insure success in our effort to "catch up with our schedule," and thereafter to issue the numbers regularly "on time." This change will involve no financial loss to any subscriber. On the contrary, Volume IV will be correspondingly larger.

As we go to press, much of the material originally put in type for this issue, that could not be included here because of the excessive bulk of this number, is in course of adjustment for the June issue, copies of which will certainly be distributed before the end of that month.

The result of a recent friendly "scrap" in the Amer. Biochem. Society shows that the Constitution of that organization is not a "mere scrap of paper," and that the society's form of government continues to be unusually democratic in character.

The following is quoted from Article V of the Constitution of the Amer. Society of Biol. Chemists:

Section 2. COUNCIL.—A. The four officers and three additional members to be elected shall constitute the Council.

B. *No two members of the Council may be from the same institution.*

Section 3. NOMINATING COMMITTEE.—Nine members *from nine different institutions* shall comprise the Nominating Committee.

Last November a number of members of the Society endorsed a proposal to amend Sect. 2 (above), by striking out sub-sect. B. The chief reason for this proposal was the opinion that the restriction (Sec. 2, B) "is unnecessarily troublesome in the selection of members of the Council." This reason would have been sufficient to commend the proposed amendment, if more important considerations had not stood in the way. Some of those who disagreed with the proponents of this suggestion considered that the adoption of the proposed amendment would facilitate the selection of any number of the seven members of the council *from any one group of workers*, and thus would discourage continuance of the present democratic and highly satisfactory method of selecting officials from widely scattered groups of workers ("institutions"). One of those who formally opposed the adoption of the proposed amendment wrote (in part) as follows, in an *open letter* to the members of the Biochem. Society:

The executive affairs of the Society should obviously be in charge of the *most representative members*. The Society is conducted, on the representative principle, for the benefit of the many and not of a few. To urge that "the executive conduct of the Society should be in the hands of the *ablest* members" is to say, in effect, that a small number of the most eminent members of the Society, in two or three centers of activity, should be continuously in charge of its management; and to express belief, besides, that they would give to such executive work the time and attention the duties deserved. Yet everybody knows of the invincible apathy among our ablest investigators regarding activity in behalf of the scientific societies. . . . The "ablest men" are usually the most indifferent to the practical activities of the societies of which they are members. Their time is "too valuable" to be wasted on "executive trifles." The constitutional requirement which it is proposed to amend, tends to place the ablest and most worthy of the younger and more active members in the official positions—the members most competent, usually, to *voice the prevailing sentiments*

and attitude of the Society at large, on practically all questions. When extraordinary occasions arise, surely the Society and the Council can easily obtain the advice and follow the guidance of the most eminent members of the Society.

The "institutional restriction" in Section 2, B—the exciting cause of the proposal to amend—was a restriction intended to distribute official *representatives* among separate and independent *groups of members*. The word "institution" was *conveniently* used in this particular sense during both the formal and informal discussions prior to the adoption of the Constitution, and has been generally so understood (as a *constitutional convenience*) since then, by all except a few who have professed to regard it as an artificial and disquieting distinction. I see in it no more danger to the delights and serenity of inter-institutional amity than in the stereotyped legend: "From the Biochemical Laboratory of Blank University, U. S. A.," which conventionally emphasizes institutions and sub-institutions as important considerations in biochemical activity and acknowledgments.

The requirement that "no two officers may be from the same institution" effects the **maximum degree of distribution** of executive *representation* and *responsibility*, *professional honors* and *service*, and *personal influence*. The most *representative* consensus of opinion and action on any subject may be expected from a body thus selected.

The highest degree of official **efficiency**, from the *representative* executive standpoint, has been an outcome, from the beginning, of the elections of officers—in short, *the restriction has worked admirably from the standpoint of efficiency*.

When the Constitution was originally presented to the Society, in 1907, it was publicly said in its support, by the same writer, with practically unanimous approval by the members:

It is aimed to make the Society thoroughly democratic and to prevent retrogression into a decadent executive system with a complacent "we-are-the-people" group at the top. Officers would be *deliberately* nominated by a large *elected* committee with that very special duty to perform, and elections could not be farcical. Automatic rotation in office at short intervals would prevent embarrassment in changing officials, good or bad; it would constantly distribute the opportunities and occasions for usefulness as well as the honors, and *thus no member would be given fictitious importance*. Adoption of the plan of distributing the officials among different laboratories would regularly insure perfectly representative composites of executive opinion and action, and would do much to prevent factionalism.

A copy of the proposed amendment was sent to each member of the Society in time for due consideration before the annual meeting in St. Louis last December (p. 182). Every member, whether present at the meeting or not, was free to cast a ballot for or against the amendment. The Secretary's public statement regarding the result of the vote, as printed in the Proceedings of the Society, announces that "*the amendment was lost.*"*

Our convictions support completely democratic *management* of scientific societies, whatever their special scientific objects or their methods of election to membership. We believe it to be in the interest of the advancement of science to oppose all non-democratic tendencies or influences in the *executive conduct* of scientific societies.

We desire to call special attention to Dr. Auer's remarks (page 186) on the undesirability of holding important scientific meetings during the Christmas holidays. He voices the earnest sentiments of many of his colleagues in serious objection to the infringements upon the domestic, social, religious, and vacation interests and preferences of scientific men, which attendance at out-of-town meetings at that particular time of year entails. Future issues of the BIOCHEMICAL BULLETIN will present further comment on this subject.

The writer has had charge informally of the editorial section of the BIOCHEM. BULL., from the beginning. Contrary to editorial customs regarding anonymity, but in full accord with scientific ideals pertaining to truth and reliability, the editorials in this journal will hereafter be published under the avowed authorship of a responsible editor, or of accredited contributors. It is proposed to extend, in the near future, the scope of the editorial department of the BULLETIN. Our new policy of fixing personal accountability for the contents of the editorials may be expected uniformly to insure a sense of responsibility, and to impose the precautions affecting credibility, that might not always obtain under the less exacting conditions of editorial impersonality.

* Personal communication by the Secretary to the writer.

The BIOCHEMICAL BULLETIN promptly acknowledges here the receipt of publications presented to it. Reviews are matter-of-fact statements of the nature and contents of the publications referred to, and are intended *solely to guide possible purchasers*; the wishes or expectations of publishers or donors of volumes will be disregarded, if they are incompatible with our convictions regarding the interests of our colleagues. *The sizes of the printed pages are indicated, in inches, in the appended notices.*

Edema and nephritis: A critical, experimental and clinical study of the physiology and pathology of water absorption in the living organism. 2d ed. By Martin H. Fischer, Eichberg prof. of physiol., Univ. of Cincinnati. Pp. 695—4x6½; \$5.00. John Wiley and Sons, New York, 1915. "These pages give in combined form the contents of the 1909 Hatfield prize essay of the Coll. of Phys., of Phila., and of the 1911 Cartwright prize essay of the Alumni Assoc. of the Coll. of Phys. and Surg., Columbia Univ., previously published as separate volumes bearing the titles 'Edema' and 'Nephritis.' The close association between the two made their appearance in combined form seem advisable. The chief changes which time has rendered necessary consist of additions to the general text embodying the results of later experimental and clinical observations in good part not readily accessible to English readers—the main argument remains as before."

Among the changes in the treatment of these subjects is an added section on the relation of syneresis to the accumulation of fluid in body cavities in edema (pp. 240-2). Special attention is also given to the possible influences of enzymes as factors in the causation of edema (p. 220). Referring specifically to the rôle of acid, Fischer says: "I have never held an acid production and accumulation to constitute, of necessity, the only factor responsible for the increased hydration which characterizes edema" (p. 220). "My constantly reiterated claim that certain changes in tissues are due to an 'increased acid content' cannot at will be made to read an 'increased (hydrogen ion) acidity.' The latter may under otherwise constant conditions become evidence of the former, but the reverse need not follow" (p. 633). Recent criticisms by Henderson and collaborators are considered on pages 633-4. This book deserves to be studied by every investigator of problems involving the biological relationships of water, for it presents effectively, and in a stimulating and interesting manner, from many points of view, the gist of our knowledge, theories, doubts and errors on this important general subject.

A review of the literature of phosphorus compounds in animal metabolism. By E. B. Forbes and M. Helen Keith, Wooster, Ohio. Pp. 748—4½x7¼. Ohio Agric. Exp. Sta'n Technical Series, Bull. No. 5. This comprehensive review is recommended unreservedly as the best work of reference on phosphorus in its relation to normal animal nutrition. The parts (pp. 13-588) are (1), Chem. of organic compounds of P; (2) P of foods; (3) P of animal bodies and products; (4) normal P metab.; (5) P metab. in disease. An unusually complete bibliography is appended (pp. 589-709), including the title of each paper mentioned; and a complete and detailed index is included (pp. 711-48). The spirit in which this splendid achievement was conceived and executed is indicated by the following quotation from the introduction (p. 11):

"Throughout the intricacies of these processes—in considering the relations of the animal to its food—let it be our point of view that inheritance has furnished the plans, the details and specifications which are to govern the whole course of metabolism; that food builds the structure and maintains its processes, in so far as made possible by the nature and amounts of its constituents; that variability in the composition and functions of the animal body, and excess of capacity in its structures, constitute a provision of safety, a means of adaptive response to changes in dietary conditions; that time lends to these adaptations such permanency, in the individual, as to constitute specific effects of foods on the life and structure of the animal; that these specific effects of foods are, in general, due rather to their limitations than to stimulation of supernormal function; that the nature and possible extent of these effects have been separately determined for each species by the particular conditions, and the *variability* of conditions of life to which, through the ages, they have become adapted; and that in relation to practical animal nutrition our interests are in the highest states of function rather than in irreducible physiological minima, since the whole range of success and profit lies close, and ever closer, to maximum possibilities."

Proceedings of the National Academy of Sciences. Arthur A. Noyes, chairman of the editorial board. The Nat'l Acad. of Sciences began, in Jan., the publication of monthly proceedings. The *Proceedings*, as the official organ of publication of the Acad., will contain reports of its business and scientific sessions, and of actions taken by its Council, notices of the Scientific and Biographical Memoirs printed for the Acad. by the U. S. gov., announcements of the awards of medals and research-grants made from its trust-funds, and statements as to other activities of the Acad. The *Proc.* will also serve as a medium for the prompt publication of brief original papers by members of the Acad. and other Amer. investigators. Its aim will be to furnish a comprehensive survey of the more important results of the scientific research of this country. Jan. issue—pp. 58— $4\frac{1}{2} \times 7\frac{1}{2}$; contains 17 papers and "report of the autumn meeting" (1914).

The chemistry of colloids and some technical applications. By W. W. Taylor, lect. in chem., Univ. of Edinburgh. Pp. 328— $3\frac{1}{2} \times 6$; \$2.00. Longmans, Green & Co., London, 1915. "It is curious that although colloid chemistry owes its development in no small degree to British investigators, hitherto there has been not only no English text-book on the subject, but no text-book in English available, the foreign works that have been translated dealing with particular aspects of the subject only, or with its bearings on other sciences." This volume is based on the author's lectures on heterogeneous systems, delivered to advanced students in the Univ. of Edinburgh. It is a very useful text-book, and a valuable work of reference for biological chemists. The four main parts deal with (1) general properties of colloids, (2) methods of preparation, (3) adsorption, (4) applications of colloid chem. (including biology, pp. 295-318).

A text-book of medical chemistry and toxicology. By James W. Holland, emeritus prof. of med. chem. and toxicol.; dean, Jefferson Med. Coll., Phila. 4th ed. Pp. 678— $4 \times 6\frac{3}{4}$; \$3.00. W. B. Saunders Co., Phila. and London, 1915. This excellent volume continues to present in systematic form the essentials of chemistry as they are related to practical and scientific medicine. The book is

primarily intended for students of medicine who are required to take, in medical schools, courses in inorganic, organic and physiol. chem., and in toxicology. In the revision successful effort was made to give a thoroly satisfactory presentation of the "aid now offered to diagnosis by the chem. laboratory." The author acknowledges his indebtedness to Prof. P. B. Hawk, and Messrs. M. A. Saylor and Olaf Bergeim, "for helpful suggestions in this revision."

Données numériques de biologie: Biochemie, chimie physique biologique, physiologie, microbiologie, pharmacodynamie. Par Emile-F. Terroine, maître de conférences de physiologie physico-chimique à l'École des Hautes-Études (Coll. de France). Préface de M. E. Roux, directeur de l'Inst. Pasteur, member de l'Inst. Introduction de M. le Dr. Delezenne, prof. à l'Inst. Pasteur, membre de l'Acad. de Médecine. Extrait du Vol. III; 1912. Pp. 20—7x8½; \$0.80. *Univ. of Chicago Press, Chicago, 1914.* Standard biochemical and biophysical data. A portion of the "*Tables annuelles de constantes et données numériques,*" published, under the auspices of the *Assoc. internationale des academies*, by the internat'l commit. appointed by the Seventh Cong. of Applied Chem. (London, June, 1909), in which the U. S. is represented by Drs. W. D. Bancroft, E. C. Franklin, H. G. Gale, G. F. Hull, G. N. Lewis, A. P. Mathews and Julius Stieglitz.

Chemical composition of the blood in health and disease. By Victor C. Myers and Morris S. Fine, prof. and lecturer, resp., in patholog. chem., N. Y. Post-Grad. Med. Sch. and Hosp., with the collab. of C. V. Bailey and F. D. Gorham, of the dep't of med., N. Y. Post-Grad. Med. Sch. and Hosp. Pp. 35—4x7. Arthur H. Crist Co., Cooperstown, N. Y., 1915. A concise discussion of "the value of various blood determinations both from the standpoint of intermediary metabolism and that of med. diagnosis," with an excellent illustrated description of methods for the determination of total nitrogen, non-protein nitrogen, urea nitrogen, uric acid, creatinin, creatin, cholesterol, "blood sugar," chlorids and total solids.

Laboratory manual for the detection of poisons and powerful drugs. By Dr. Wm. Autenrieth, prof. in the Univ. of Freiburg i/B.; transl. (4th German ed.) by Wm. H. Warren, prof. of chem., Wheaton College. Pp. 320—4x6¾; \$2.00. P. Blakiston's Son and Co., Phila., 1915. This volume maintains its high reputation for convenience and reliability as a lab. manual for students, and as a guide for toxanalysts. The thorough revision to which this edition has been subjected has increased its size, scope and value, without any loss of its familiar excellences. The translator has added several important features, such as a very effective discussion of "normal arsenic."

A laboratory manual of qualitative chemical analysis, for students of medicine, dentistry, and pharmacy. By A. R. Bliss, prof. of chem. and pharm., Birmingham Med. Coll. Pp. 244—3¾x6¾; \$2.00 net. W. B. Saunders Co., Phila., 1914. The course of instruction presented in this manual presupposes that the student begins with a fair degree of knowledge of the principles of chemistry. The directions are clear and accurate; the arrangement, logical. The book is particularly well suited to the needs of the types of students for whose use it was prepared.

A civic biology, presented in problems. By Geo. W. Hunter, head of the dep't of biology, De Witt Clinton High Sch., N. Y. City. Pp. 432—4x6; \$1.25.

Amer. Book Co., 1914. "This book shows boys and girls living in an urban community how they may best live within their own environment and how they may cooperate with the civic authorities for the betterment of their environment." In the attainment of this purpose the book is a notable success. The chem. of foods, and the chem. physiol. of plants and animals, receive special attention and are treated in very interesting and instructive ways.

A study of foods. By Ruth A. Wardall, head of Dep't of Home Econ., State Univ. of Ia., and Edna N. White, head of Dep't of Home Econ., Ohio State Univ. Pp. 174— $3\frac{1}{2} \times 5\frac{1}{2}$; 70 cents. Ginn and Co., 1915. The subject is presented clearly and accurately in a simple and concise manner, from the standpoints of the general nature of food materials, effects of heat upon them, methods of manipulation, and comparative cost of commercial and domestic products. The book is particularly valuable for use in "food courses" in secondary and extension schools.

Merck's chemical reagents, their purity and tests. 2d ed. (Authorized transl. of Prüfung der chemischen Reagenzien auf Reinheit, 2te Aufl., von E. Merck.) By Henry Schenck. Pp. 199— $4 \times 6\frac{1}{2}$; Merck and Co., N. Y., 1914. This well known volume has been improved by the inclusion of many new subjects. The tables of equivalents of standard solutions have been replaced by a table giving approximate strengths and brief directions for the preparation of solutions for reagent purposes, compiled from published data.

Chemical and biological survey of the waters of Illinois. By Edward Bartow, Univ. of Ill. Bull., Vol. X, No. 36, 1912. Water survey series, No. 10; Pp. 198.—*Idem.*, Vol. XI, No. 38, 1914. Water survey series, No. 11; Pp. 478.

Pellagra II. Second progress-report of the Thompson-McFadden Pellagra Commis. of the N. Y. Post-Grad. Med. Sch. and Hosp. By J. F. Siler, captain, Med. Corps, U. S. Army, P. E. Garrison, passed assist. surgeon, U. S. Navy, and W. J. MacNeal, assist. director of lab's, N. Y. Post-Grad. Med. Sch., with the collab. of H. Douglas Singer, Paul A. Schule, O. S. Hillman and others.

Researches in biochem., conducted in the Johnston Lab., Univ. of Liverpool. Ed. by Benj. Moore, Johnston prof. of biochem., Univ. of Liverpool. Vol. III; 1912-'14. (20 reprints.)

Collected papers: Inst. of Physiol., Univ. College, London. Ed. by E. H. Starling, Jodrell prof. of physiol. Vol. XVIII, 1913-'14. (25 reprints.)

Studies from the Otho S. A. Sprague Memorial Inst. Vol. II; 1914. (30 reprints.)

Studies from the Rockefeller Inst. for Med. Research. Vol. XX; 1915. Pp. 591. (55 reprints.)

Studies from the Dep't of Physiol., Cornell Univ. Med. Coll., Vol. III; 1914. (21 reprints.)

Studies from the Dep'ts of Pathol., Bacteriol., Exp. Pathol. and Exp. Therapeutics, Cornell Univ. Med. Coll., Vol. XIII; 1913. (21 reprints.)

Collected papers, 1913-1914: Lab. of Physiol. Chem., Sheffield Scientific School, Yale Univ. By R. H. Chittenden, prof. of physiol. chem., L. B. Mendel, prof. of physiol. chem., and F. P. Underhill, prof. of patholog. chem. (42 reprints.)

Biochemical Research, 1912-13: By the staff of the Biochem. Lab., Univ. of Chicago. Albert P. Mathews, editor. (20 reprints.)

Contributions from the Physiol. Lab. of the Medico-Chi. Coll., Phila.: Part XX of Ott's contributions to physiol. By Isaac Ott and John C. Scott. 1914. (9 reprints.)

A text-book of physiological chemistry. By Albert P. Mathews, prof. of physiol. chem., Univ. of Chicago. Pp. 700. Wm. Wood and Co., N. Y., 1915. [In press.]

Chemical pathology. Being a discussion of general pathology from the standpoint of the chemical processes involved. By H. Gideon Wells, prot. of pathology, Univ. of Chicago and Rush Med. Coll., and Director of the Otho S. A. Sprague Memorial Inst., Chicago. 2d ed. Pp. 616—4¼ × 7; \$3.25 net. W. B. Saunders Co., Phila., 1914. The second edition of this invaluable text book fully meets the expectations of those of us who have constantly used the first edition (issued in 1907). It is impossible to suggest the merits of this splendid volume in notes as brief as these without the use of superlative terms in every line of comment. Written by one who has been thoroughly trained in pathology and biological chemistry, and whose experience as a teacher and investigator of the chemical aspects of pathology has been exceptional, this volume by Wells presents in masterly manner the essentials of chemical pathology from every standpoint of importance. Practitioners of medicine, expert pathologists, laboratory workers in every medical school and institute, biological chemists everywhere, and biologists in general, will find this book of exceptional utility. A biochemical laboratory cannot be up to date without it.

Chimie pathologique tropicale de la région Atlantique. By G. Delgado Palacios, prof., Univ. of Caracas, Venezuela. Pp. 318—4¼ × 6¾. Published by the author, 1914. A special treatise on feces, intoxications of intestinal origin, nutritional disturbances due to intestinal influences, and intestinal disinfection, especially from the standpoint of nutrition in the tropics. The author describes the calcareous product from feces of inhabitants of the tropics, called "carcoma fécale," which occurs in the form of granules 0.1-0.3 mm. in diameter, and contains unrobilinogen and another chromogen termed *cholérythrogène*—a name intended to suggest the origin of the new chromogen and the color of its pigment derivative. The origin, significance and properties of "carcoma fécale" and cholérythrogène, and their relation to yellow fever and tropical pathology, are discussed in detail. The book concludes with a section on biochemical methods of general value. The author is now in this country and may be addressed "in care of the BIOCHEMICAL BULLETIN."

The nature of enzyme action. By W. M. Bayliss, prof. of general physiology, Univ. Coll., London. 3d ed. Pp. 180—4¾ × 7½; \$1.50 net. Longmans, Green and Co., London, 1914. (One of the *Monographs on biochemistry*.) The most valuable book in English on enzymes. The author has incorporated the gist of the many recent discoveries on reversibility, on combination between enzyme and substrate, and on anti-enzymes. The chapters on these subjects have been rewritten, for the most part, and the whole book brought up to date.

MEMBERS OF THE COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

Official register: Edited by the Secretary,

EDGAR G. MILLER, JR.¹

Honorary Members

- PROF. R. H. CHITTENDEN, *First Director of the Columbia University Department of Biological (Physiological) Chemistry and Director of the Sheffield Scientific School of Yale University*
- *PROF. HUGO KRONECKER, *Director of the Physiological Institute, University of Bern, Switzerland. (Died, June 6, 1914.)*
- PROF. SAMUEL W. LAMBERT, *Dean of the Columbia University School of Medicine*
- DR. JACQUES LOEB, *Member of the Rockefeller Institute for Medical Research and Head of the Department of Experimental Biology*
- DR. SAMUEL J. MELTZER, *Member of the Rockefeller Institute for Medical Research and Head of the Department of Physiology and Pharmacology*
- PROF. LAFAYETTE B. MENDEL, *Professor of Physiological Chemistry, Sheffield Scientific School, Yale University*
- PROF. ALEXANDER SMITH, *Head of the Department of Chemistry, Columbia University*

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THE DEPLORABLE CONTRAST BETWEEN INTRANATIONAL AND INTERNATIONAL ETHICS, AND THE MISSION OF MEDICAL SCIENCE AND MEDICAL MEN*

S. J. MELTZER

The chief aim of my remarks is to point out the unique position which medical sciences and medical men occupy in the horrible war which is going on now between civilized nations. International morality may possibly derive some permanent benefit from a conscious knowledge of this position. However, in order to make my point clear, I shall introduce it by a general discussion of some aspects of ethics.

Moral philosophy assumes for granted that ethical relations of civilized men are safely established; it concerns itself merely with the question regarding the nature of the *origin* of ethical precepts. In general, it may be admitted that the vast majority of civilized men indeed do not question the correctness of ethical demands. But writers on moral philosophy fail to distinguish between *intranational* and *international* ethics. Hence, we find frequently that international occurrences are discussed from the point of view of intranational principles; international occurrences are brought before the forum of a supreme court of the world for judgment, but the merits and demerits of the cases argued from the point of view of ethics which obtain in intranational moral relations. But the truth is that there is an abyss between the two domains of morality.

* Address delivered at the fourth annual dinner of the Columbia University Biochemical Association, March 26, 1915.

Let us first look at the status of intranational morality. The ethical relations among civilized fellow-men, united by bonds of race, nation or country, are firmly established. Justice and duty are deeply rooted conceptions, the compelling force of which is spontaneously recognized by all normal members of the individual community; the small fraction of dissenters consists of defectives and criminals. Sympathy, kindness, altruism and self-sacrifice are not enforceable human virtues, but are nevertheless profoundly appreciated and admired by the individuals of all civilized nations. *Honesty* is an indispensable virtue. In parenthesis I may, however, say here that to my knowledge "honor" is not among the general precepts of ethics. It is an artifact; it is mostly an artificial virtue of a class which considers itself as being above the simple requirements of justice and duty. It is not an unusual occurrence that in the name of honor a man may slay with relative impunity a fellow-man whose home life he has dishonored.

From Sokrates to our day students of moral philosophy offered various theories concerning the nature of the principles underlying the "science of conduct." I shall not discuss the merits of the theories of Hedonism or Utilitarianism, the Law of God or the Categorical Imperative; they do not concern us here. But I have to refer to one theory which was not received with great favor and which had only a short life of popular existence. In the latter half of the last century, under the powerful influence of Darwin's theory of natural selection in the domain of biology, a systematic attempt was made by some philosophers (Herbert Spencer and others) to look upon ethics as a purely biological phenomenon. Family ties of lower animals, it was thought, developed into the ethics of civilized nations. Whether on account of the feverish social and altruistic activities which have been going on in the last decade or two and for which a biologic theory of ethics could hardly have served as a sufficient stimulus; or whether on account of the general decadence in popular enthusiasm for the theory of natural selection in general, the fact is that the theory of biologic origin of ethics seems to have been generally abandoned in recent years. But whatever we may think philosophically regarding the *nature of fundamental origin of ethics*, we can not deny that *morality*

is subject to evolutionary influences; it has undergone and is continually undergoing development. Morality manifests a continuous growth. The development of savage races into cultured, ethical nations is a matter of historical record. In fact, the progressive widening which conceptions like justice or duty are continually undergoing within the confines of a nation is practically a matter of direct observation during an individual's lifetime.

I shall dwell here especially on two elements which are operative in this process. The foremost factor in the evolutionary progress of intranational morals is to be found undoubtedly in the intellectual activities peculiar to man. The growth and development of the sciences, of arts, music, poetry, literature and religion, from their rudimentary phases into their present high states, elevated the specific human character and favored the widening and deepening of morality of any individual nation or rather the morality of the individuals of which these nations are composed. The human intellect may or may not be the primary cause of morality; but the unfolding of human intelligence and the growth of intellectual activities specifically human, are undoubtedly important elements in the growth and development of specific human morality. This connection between intelligence and morality is practically a matter of direct observation.

On this basis the further assumption is justified, that even the conscious primitive morality of primitive man did not make its appearance abruptly. It developed very slowly, parallel, to a certain degree, with the development of man in the animal stage into man with rudimentary intelligence.

I presume, then, that conscious morality did not begin abruptly, but developed very slowly, parallel with and assisted by the development and growth of human intelligence. However, important as the human intelligence may be, evidently it is not the only controlling factor of morality. We see animals acting towards their fellow-creatures in a manner which, if seen in human beings, we would consider as highly ethical. We all know how animals care for their offspring. We see dogs licking the wounds of their fellow-dogs—an act resembling a samaritan service. We see altruistic activities in the communities of the bees and the ants. We desig-

nate these animal activities as instincts and we have indeed no evidence that a conscious morality is at the bottom of these phenomena. We have, however, to keep in mind that the harmonious relations between animals are observed only among individuals of the same species or race, or the same drove or swarm, whether they are presided over by a bell-wether, a queen or any other single leader, or have a democratic form of government with several contending leaders. Animals belonging to different species, races or strains get frequently into ferocious fights as soon as they meet, or as soon as there is a collision of interests and instincts. There are therefore sufficient reasons for assuming that the purely animal, instinctive element is involved to a considerable degree in the moral relations between individuals of the same group of human beings which have some efficient bond in common.

Now let us look at the moral aspects which international relations present. The history of nations, civilized or uncivilized, consists chiefly of a tale of more or less ferocious wars interrupted by periods of peace. War is nothing but wholesale murder; but the men of one tribe or nation who are murdering men of another tribe or nation have no idea that they are committing crimes; on the contrary, the more civilized individuals among the fighters are honestly possessed by the conviction that they are performing a moral duty. It is true that in times of peace citizens of one country enjoy in another country most of the privileges enjoyed by the citizens of that country. This is guaranteed by treaties. There are also international laws which even presume to prescribe the mode of warfare among the signatory powers. In time of peace a sincere friendly intercourse frequently prevails between the individuals of various nations. There are numerous international reunions for the purpose of furthering human knowledge and general human interests in all lines of human endeavor. All these facts may give us the right to speak of international morality. Nevertheless, even peace, especially peace in modern times and among civilized people, is practically nothing more than a *truce* during which nations are feverishly active in preparing for the next war, preparing to slaughter their apparent friends of to-day and to lead or to drive their own men to be slaughtered. During peace the leaders of nations are

engaged in their military quarters or in their chancelleries in spying upon and intriguing against the nations with whom they exchange international amenities.

In international dealings cunning and deceit are essential factors in success; it is diplomacy. *Honesty has hardly a place in these dealings. Only honor is the big word which is loudly used by those who speak for nations as units, that sham virtue in the name of which crimes are committed by the privileged classes within each nation and in the name of which hundreds of thousands of honest and innocent citizens of various nations are murdered or crippled for life in the groundless and senseless strife of nations, brought about by the ambitions of unprincipled leaders.* Furthermore, international relations in time of peace, which have an ethical appearance, are held together by flimsy ties. International peace conferences, international law, and peace treaties are merely scraps of paper which are torn to shreds at first sight of a bone of contention between nations.

In a previous section I insisted, and I believe rightly, that intellectual growth and activity are most important factors in the development and growth of *intranational* morals. What is the value and influence of intellectual growth and activity in *international* morals? Highly intellectual, civilized nations fight one another with a rage, a ferocity and with an intent to kill as probably did their animal ancestors of different strains or races, hundreds of thousands of years ago. But different species of another type of animals, let us say dogs and cats, are probably fighting to-day as their ancestors fought thousands of years ago, that is, tooth and nail, the only weapons at their disposal; their physical agility, their promptly acting reflexes, the finer developed senses and their remarkable instincts did not help them in developing new weapons or new ways of fighting; they had no human intellect. But the human race? We need not go back thousands of years. It suffices to compare warfares separated only by a hundred years. I need not enter upon a comparison of the rage, brutality and barbarity with which the wars are conducted; in this regard the present war is surely not behind its predecessors, and none of the cultured belligerent nations is ahead of or behind the others. But as to destructiveness of human

life, that cardinal aim in the war of nations, the progress made in this comparatively short span of human history is immense; it reads like a fairy tale. From high in the air a human bird directs you to turn a micrometer screw one millimeter or two and a huge shell annihilates hundreds or thousands of your enemy. A small group of human fishes bubble up in the vicinity of a huge leviathan, a dreadnought, and in less than ten minutes hundreds of men and millions of dollars are forever at the bottom of the sea. In a stretch of hundreds of miles, hundreds of thousands of soldiers are moved rapidly without a hitch from one place to another where they are needed most. The success is wonderful. In barely eight months millions of people were killed or crippled, perhaps as many more were made homeless and driven into starvation, and billions of dollars borrowed and wasted. And that astounding result was not accomplished as in olden times, merely by extraordinary physical force or endurance or by that virtue in which wild beasts greatly excel men, the virtue of physical courage; it was accomplished by specific human ingenuity. Mathematics, physics, chemistry and other theoretical and practical sciences have made these awful results possible. In fact, practically every kind of intellectual activity took and takes a profound part in the bitter struggle which now goes on among highly civilized nations. Historians, philosophers, literary men and others are busy contributing offensive and venomous literature about their fellow-men of nations with whom their country is at war, whose friends they were and whose honors they enjoyed. Poets sing the song of profound hatred and musicians write the melody to it, or compose war marches and songs. Religion offers an extraordinarily sad spectacle. Nations having the same religion and believing in the same God, pray to Him that He may help them destroy their enemy. Think of the robber and murderer who on his most godless errand prays to God for aid and guidance!

But here I must call your attention to a paradoxical but remarkable fact. Beastly as international morality is, when nations are at war, war nevertheless unquestionably elevates the *intranational* morality. The majority of citizens in every country are not idealists; in time of peace they comply with the laws of their country

and fulfill their simple duties, not more and not less. But when their country is at war, a new spirit comes over them; they become altruists, they are ready to bring sacrifices, to lose their lives or to become cripples for life. Whether a country is right or wrong with regard to the merits of a particular war in the eyes of an outsider, a neutral, this has no bearing upon the moral status of the man in his own country. That status is unquestionably elevated during war, and even after the war his relations to his countrymen remain on a higher moral plane.

Now let me recapitulate briefly. Human morality, whatever the nature of its origin may be, was and is subject to evolutionary influences. It began in the pre-savage state of men. Its development has been and is a very slow process. In its present state we must sharply distinguish between intranational and international ethics; there is an abyss between them. Intranational morals attained a high state. Intellectual activities of all kinds were and are most important factors in its growth. The morality in international relations, on the other hand, is generally low, and is frightfully bad when these relations are interrupted by war. War is an animal method of settling differences between two contending vicious species, and human intellectual activities greatly intensify the deadliness of the procedure. The efforts to create international laws for the purpose of restraining the ferocity of international struggles proved of little avail. We have cultured, civilized Germans, Frenchmen, Englishmen, and so on, *but the world is not yet inhabited by cultured civilized men.*

Apparently biological processes are operative in these horrible differences between the intranational and international states of morality. Intellectual activity is capable of efficiently assisting in the development of morality among individuals which are allied by some organic and social bonds; thus little or no resistance is offered to the beneficent intellectual influence. But individuals of different strains, with natural divergences and antagonisms, sustained by differences in education, customs, forms of law, etc., offer great resistance to the unifying influences of intellectual activity.

Accordingly, biological traits common to all animals, while some of them may exert a favorable influence upon the evolution, rate of

growth and the direction of human morality, are surely not the main factors of its creation and development. On the contrary, in interracial and international relations many biological traits are profoundly inimical to a development of proper moral ideals. Struggle for existence, physical strength and dexterity, love of fight, hate, rage, bravery, etc., are traits which the human race has in common with wild beasts, and an uncontrolled cultivation of these traits may often prove disastrous to all human morality. On the other hand, intelligence and intellectual activities are traits which distinguish man from beast. Their intense cultivation by civilized men has been the main cause of the high state of morality which prevails and is visibly progressing within the confines of civilized countries—the *intranational* ethics.

But now let us turn again to *international* ethics. We have seen that there is an abyss between international and intranational morality. We have seen further that war between civilized countries brings in modern times incomparably more frightful results than in previous ages, which is undoubtedly due to the astounding discoveries and inventions brought to light by the intense intellectual activities in the various cultured countries. Are discoveries and inventions, are even apparently sound intellectual activities, dangerous to international morality? Is this morality rather regressive instead of being progressive? And what can we do to make it progressive or to accelerate the imperceptible progress? The last question is the more important one, since it presents a practical and not merely an academic problem. In the following I intend to discuss some factors which may contribute in some modest way to its solution. I am fully aware, as all of you are, of the immensity of the problem, and I am aware, more than you, of the microscopical dimensions, metaphorically speaking, of your guest of the evening. But I shall act now as I always acted, upon the principle that it is neither good nor wise to possess less courage or more modesty than that drop of water which innocently and cheerfully undertakes to drill a hole in a rock.

As one who swore allegiance to the medical tribe, I shall begin by saying that the case of international morals is very bad indeed, but it is by no means hopeless; that only hopeful men are capable

of attaining desirable results; that a remedy which promises to bring some help, be it ever so small, is not to be despised, and that a sum of such remedies may save even a bad case.

It seems to me quite probable that interracial and international morals are also subject to evolutionary influences and are undergoing a developmental process; but *the progress is extremely slow because it has to struggle against the beastly nature of man*. Even the development of intranational morality is a slow process; it must have taken many thousands of years before it reached its present stage. The present condition of international ethics would perhaps appear to us even quite high, if we had the means to compare it with its status of hundreds of thousands of years ago. This recognition, namely, that interracial and international morals are undergoing a progressive development, but that their progress is necessarily very slow, seems to me to be a very useful one; because it encourages us to try to accelerate this progress, be the rate of the possible increase in the acceleration ever so small and be the means at our disposal for accomplishing it ever so meager.

I do not consider it as my province to try to discuss here all sorts of means which possibly may serve to increase progress in international morality. My chief purpose is, as stated at the beginning, to bring forward the value of medical sciences and medical men as efficient factors in furthering the progress of international morality. However, before coming to it, I wish to call attention briefly to a point or two to which reference has been made before. I believe, in the first place, that it is of prime educational importance to point impressively to the fact that there is a gulf between national morality, on the one hand, and interracial and international morality, on the other hand. A confusion between the two sets of ethics may harm the former and retard the possible progress of the latter. Citizens in neutral countries at all times, and citizens of all countries in times of peace, should know, should feel it deeply in their hearts, that war has not the slightest feature of morality, that it is simply a mode of settling differences between two or more strains of the human race in the fashion of wild beasts, increased in deadliness and ugliness by the activities of human intelligence. Here is an incontestable fact which gives pain and distress to the moral man;

humanity, as a whole, shows that its moral conduct is not above that of vicious animals of various species. The discussion of the question as to who began the war and who prevents its conclusion is far from the mark; it is purely academic and is borrowed from the point of view of intranational morals. Justice and law had little to do with the beginning of the war and will have very little to say with its settlement. War is carried on by brute force and is settled by it with the aid of exhaustion and starvation. The many circumstances which lead to the numerous wars are mere incidents, but not the real cause of them. There is only one cause for all the wars and that is the possession by human beings of ferocious qualities peculiar to wild beasts, often entirely unrestrained and sometimes even directly cultivated to a higher degree.

In teaching intranational morality it ought to be made clear that physical strength, courage, dexterity and efficiency, useful and desirable as they are for the success in the life of the individuals and the nation they compose, are not moral principles. On the contrary, they may greatly magnify the evil results when used for unethical principles. Bravery and efficiency, which are most highly valued qualities in war, are qualities which are most destructive to your so-called enemy of to-day and perhaps your friend of yesterday and, moreover, perhaps of your friend of a day after to-morrow.

I now come to the chief point I wish to discuss. Short as the discussion will be, it is nevertheless the chief object of my entire discourse. I have stated above that the striking feature of this war, the great destructiveness of human life, owes its success to the employment of scientific results in carrying on the war. All sciences which may contain some practical element are contributing in some way or another to the wholesale destruction of human life. And not only the scientific results, but the scientists themselves are active at the front in laboratories improvised in large automobiles to search for new inventions and discoveries which may be of some immediate practical use or to predict the nature of the weather to be expected at different points, etc. *And those who can not assist in such a direct way try to contribute to the spirit of war by spreading enthusiasm, by abusing the enemy, and by implanting hatred against it.*

But there is one most inspiring exception to this sorrowful rule. It is the utilization of the medical sciences and the behavior of medical men in the war. The results of medical investigations of the last few decades and the activities of medical men are of immense practical importance to modern warfare. In some of the former wars perhaps as many soldiers were wiped out in consequence of disease as were killed by the bullet or bayonet. The combined modern studies in pathology, bacteriology, hygiene, surgery, medicine, pharmacology, preparation of antiseptics, etc., have immensely reduced the ravages of war as far as sickness and injuries are concerned. Medical sciences and medical men are part and parcel of wars. But what is their ethical status with reference to strife of nations in comparison with other sciences, with other men of science, men of culture and education? Here is the answer.

None of the numerous important discoveries made in the medical sciences was ever used for the destruction of life or harming the enemy in modern civilized warfare.

Any discovery or invention made in the sciences or the practise of medicine, made in one of the warring countries, is freely given to the medical fraternity of a belligerent country—unless it involves a business relation over which medical men have no power. It is illuminating to read a review in an English medical journal of medical reports made at a German medical meeting held on a battlefield.

On the battlefield, on the firing line, perhaps in the midst of a hail of bullets and fragments of shrapnel, *physicians and surgeons, some of them volunteers, pick up wounded soldiers without regard to nationality, and treat friend and foe alike. It is practically of no moment to the sick and wounded soldier to which of the hospitals of the civilized belligerent nations he will be taken for treatment.* The physician, as a physician, knows no difference between races and nations, between friend and foe.

And withal physicians in every one of the warring countries are as good patriots, and are as ready to sacrifice their lives in their country's struggle, as any other patriotic citizen of his beloved country, with the only difference that he, *the physician, is merely ready to die, or to be crippled for life, in the service for his country,*

but he is not engaged in killing or harming any one belonging to another nation or country.

There might be a few exceptions—it would be miraculous indeed if there would be none; any large group has its exceptions. But such few exceptions can not be held up against this wonderful picture which medical men present in war. And wonderful indeed this picture is. We have seen how low international morality is at all times; we see how infamously bad it is in time of war and especially in the present ferocious war of cultured nations. And in the midst of this inferno we perceive a group of sciences which are in intimate contact with life and with war, and which nevertheless never contribute to the degradation of interracial or international morality. We perceive, furthermore, in every belligerent nation among the combatants a group of patriotic men, brave and ready for every self-sacrifice, who do nothing but render help to those who need it, who render it as members of their particular country, but render it to foe and friend alike. Here are representatives of humanity, as a whole, here is a most encouraging example of an elevated international morality.

This wonderful fact is not my discovery; it is a fact well established, and well known to everybody, at least ought to be known by everybody. *But the calling of this fact to full consciousness in the members of our profession may render a great service to the progress of international morality.*

In the dawn of history, the medical man was also the exponent of philosophy and morals. In the middle ages when knowledge became specialized, medical men more and more devoted their activity exclusively to medical practise. On account of the inefficiency of medicine at that time, medicine lost its prestige. Recently, however, medicine became a science and one marvelous discovery follows another; and the efficiency of medical practise increases rapidly. Medicine makes inhabitable to man hitherto uninhabitable parts of the world. It prevents disease, and with increased efficiency it learns to cure it. Medical sciences and medical men have steadily risen in the estimate of discriminating civilized mankind. *Could medical sciences and medical men not become again the standard-bearers of morals, especially of international morality?*

In the furious struggle which is going on at present amongst civilized nations international morals lost its friends; religion, sciences, and the brotherhood of mankind proclaimed by the followers of socialism failed it; medicine alone did not desert it. In times of peace and for the purpose of furthering useful knowledge medical sciences and medical practices are working in separate groups, according to their specific aims. But all medical men of various shades and groupings ought to unite for this one high aim, *ought to establish a Medical Brotherhood for the Purpose of Upholding and Accelerating the Progress of International Morality.*

Every one of the scientific and practical men in medicine in our large country ought to join with enthusiasm such a missionary enterprise. The initiative ought to be taken by our large neutral country, but we may appeal to our neutral brethren in other neutral countries to join our crusade. However, we must not approach our medical confreres in the belligerent nations as long as the war lasts, lest it may be interpreted as an attempt to weaken their patriotism and their enthusiasm for the cause of the particular countries of which they are integral parts.

*Rockefeller Institute for Medical Research,
New York City.*

MEDICAL BROTHERHOOD
FOR THE FURTHERANCE OF INTERNATIONAL
MORALITY

Fraternitas medicorum

F. M.

ORIGIN, ORGANIZATION, PROCEEDINGS, REPORTED BY THE FIRST SECRETARY,

WILLIAM J. GIES

At the fourth annual dinner of the Columbia University Biochemical Association (March 26, 1915), which was attended by about 200 members and guests of the Biochemical Association, and at which he was the guest of honor, Dr. S. J. Meltzer delivered the address that is published on the opening pages of this issue of the *BIOCHEMICAL BULLETIN*. In that address Dr. Meltzer proposed that "all medical men of various shades and groupings . . . ought to establish a Medical Brotherhood for the Purpose of Upholding and Accelerating the Progress of International Morality." At the conclusion of Dr. Meltzer's address the large assembly was invited to express its opinion, by a rising vote, on the desirability and feasibility of organizing such a Brotherhood. The vote was unanimously in the affirmative. (See pages 263 and 267 of this volume of the *BIOCHEMICAL BULLETIN*.)

Dr. Meltzer's address was published in *Science* (April 9, 1915; p. 515). Shortly thereafter Dr. Meltzer communicated with some of his colleagues regarding their personal willingness to participate in the organization of the proposed Brotherhood and was heartily encouraged in his plans to effect its establishment.

On July 3, 1915, Dr. Meltzer issued a statement, in part, as follows:

Dear Doctor:

It gives me pleasure to inform you that the matter of establishing a Medical Brotherhood for the Furtherance of International Morality has now reached a satisfactory stage. I am, therefore, taking steps to form a definite organization. More than 140 medical men, among them many of the most prominent and influential men in this country, have agreed to serve on the Committee that will issue an Appeal to the medical profession of this country. I enclose the list of the members of the Committee; it is probable that the final list will comprise 150 names. In response to an appeal by me, the Executive Committee of the Carnegie Endowment for International Peace has appropriated \$1500 for carrying out the preliminary work, and it is hoped that other contributions will later be received.

I am sending you a copy of the Appeal that will be sent to a large number of physicians in this country. Will you kindly read it and suggest any improvements in its wording that may occur to you.

In order to effect a promptly active organization, I suggest that you authorize me to invite New York colleagues to serve on a provisional Executive Committee of 15 members.

Please let me have your suggestions of names of such colleagues within the next ten days. At the expiration of this period we shall count the votes and the fifteen who receive a majority will be considered as constituting the Executive Committee. A meeting of these men will then be called, at which a permanent organization will be effected.

Sincerely yours,

S. J. MELTZER.

On July 18, Dr. Meltzer forwarded to all of the members-elect of the provisional Executive Committee a note inviting them to meet him at the New York Academy of Medicine for the purpose set forth in the foregoing letter.

Pursuant to Dr. Meltzer's call, as stated above, there assembled, in the Council room of the N. Y. Academy of Medicine, on Tuesday, July 20, 1915, at 4.15 p. m., Drs. S. Josephine Baker, John W. Brannan, Harlow Brooks, Rufus Cole, John A. Fordyce, Nellis

B. Foster, William J. Gies, Samuel J. Meltzer and Robert T. Morris.

Dr. Meltzer took the chair, called the meeting to order and requested Dr. Gies to serve as temporary secretary. Dr. Meltzer stated the object of the meeting to be the organization of the Medical Brotherhood for the Furtherance of International Morality, in harmony with formal authorization to that end on behalf of the 150 men and women whose votes designated the membership of the executive committee assembled at this meeting.

Dr. Meltzer then stated that the colleagues named below, who favored the organization of the Medical Brotherhood, had been designated, by pluralities of the votes cast in accordance with the terms of his invitation dated July 3, 1915 (as copied above), to serve as an Executive Committee and to proceed with provisional organization of the proposed Medical Brotherhood:

Robert Abbe	J. A. Fordyce	Graham Lusk
S. Josephine Baker	Nellis B. Foster	Samuel J. Meltzer
J. W. Brannan	William J. Gies	Robert T. Morris
Harlow Brooks	S. S. Goldwater	William H. Park
Rufus Cole	Abraham Jacobi	John A. Wyeth

It was voted that a provisional organization be effected by the election of officers to serve for a term of one year, or until the election of their successors. The following officers were then elected:

I. EXECUTIVE COMMITTEE

(Residents of the City of New York)

A. Active Officers

PRESIDENT: S. J. Meltzer, Member, Rockefeller Institute.

VICE-PRESIDENTS: Rufus Cole, Director, Rockefeller Hospital;
S. Josephine Baker, Director, Bureau of Child Hygiene, Department of Health.

FIRST SECRETARY: Wm. J. Gies, Professor of Biological Chemistry, Columbia University.

SECOND SECRETARY: Harlow Brooks, Professor of Clinical Medicine, University and Bellevue Hospital Medical College.

TREASURER: Robert T. Morris, Professor of Surgery, Post-Graduate Medical School.

B. Councillors

- Abraham Jacobi, Professor of Diseases of Children, Emeritus, Columbia University.
- Robert Abbe, Surgeon, St. Luke's Hospital.
- John Winters Brannan, President, Board of Trustees of Bellevue and Allied Hospitals.
- J. A. Fordyce, Professor of Dermatology, College of Physicians and Surgeons.
- Nellis B. Foster, Assistant Professor of Medicine, Cornell University Medical School.
- S. S. Goldwater, Commissioner, Department of Health.
- Graham Lusk, Professor of Physiology, Cornell University Medical School.
- William H. Park, Professor of Bacteriology, University and Bellevue Medical College.
- John Allan Wyeth, President, Polyclinic Hospital.

II. ADVISORY COMMITTEE**A. Honorary Presidents**

- Rupert Blue, Surgeon General, U. S. Public Health Service, Washington, D. C.
- W. C. Braisted, Surgeon General, U. S. Navy, Washington, D. C.
- Russell H. Chittenden, Director, Sheffield Scientific School, Yale University.
- W. T. Councilman, Professor of Pathology, Harvard Medical School.
- W. C. Gorgas, Surgeon-General, U. S. Army, Washington, D. C.
- W. S. Halsted, Professor of Surgery, Johns Hopkins Medical School.
- W. H. Howell, Professor of Physiology, Johns Hopkins Medical School.
- Abraham Jacobi, Professor of Diseases of Children, Emeritus, Columbia University.
- W. W. Keen, President, International Surgical Congress; President, American Philosophical Society, Philadelphia.
- Edward L. Trudeau, Saranac Lake, New York.
- James Tyson, Professor of Medicine, Emeritus, University of Pennsylvania.
- Victor C. Vaughan, Professor of Hygiene and Physiological Chemistry, University of Michigan.
- William H. Welch, President, National Academy of Sciences; Professor of Pathology, Johns Hopkins Medical School.

B. Honorary Vice-Presidents

- J. J. Abel, Professor of Pharmacology, Johns Hopkins Medical School.
Herman M. Biggs, Commissioner, State Board of Health, New York City.
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Jonathan Wright, New York City.

The officers were instructed to proceed, with all necessary or desirable measures, to enlarge the membership of the Brotherhood and to distribute, to that end, copies of the following appeal, as originally circulated by Dr. Meltzer :

An Appeal to the Men and Women Engaged in Medical Practice and the Advancement of Medical Sciences

The present horrible war among civilized nations has brought out impressively certain sad facts: that although there are civilized

individual nations, we are still very far from having a civilized humanity—there is an abyss between *intranational* and *international* morality; that, no matter how cultured and enlightened nations may be, they still settle their international differences by brute force, by maiming and killing their adversaries; and, finally, that the present high development of science and invention in individual nations only serves to make the results of this war more destructive than any other in history.

The war has demonstrated, however, one encouraging fact; namely, that among all the sciences and professions, the medical sciences and medical practice occupy an almost unique relationship to warfare, and that among all the citizens of a country at war, medical men and women occupy a peculiar and distinctive position.

No discovery in medical science has been utilized for the purpose of destroying or harming the enemy. Medical men in each of the warring countries are as courageous, as patriotic, as any other citizens, and are as ready to die or to be crippled for life in the service of their country as any other class of their fellow countrymen. Their services, however, consist in ministering to the sick and to the injured, and in attending to the sanitary needs. Furthermore, they often risk their lives by venturing into the firing line to bring the injured to places of safety and to attend to their immediate needs. *In these heroic and humanitarian acts friend and foe are treated alike.* Finally, the majority of the members of the medical profession and of the medical journals of the neutral as well as of the warring countries, abstain from public utterances that might be grossly offensive to any of the belligerent nations.

These facts—this advanced moral position in international relations which medicine and its followers are permitted to occupy in all civilized nations—ought to be brought to the full consciousness of the men and women engaged in the medical sciences or in medical practice. Such a realization could not fail to have an elevating influence upon the medical profession itself, and would probably exert a favorable influence upon the development of international morality in general.

At the dawn of history, medical men were frequently also the exponents of philosophy and morals. In the middle ages, when

knowledge became specialized, medical men more and more devoted their activity exclusively to medical practice. Because of its inefficiency at that time medicine lost its prestige. In recent times, however, medicine is becoming an effective science; one marvelous discovery has followed another, and the efficiency of medical practice has been rapidly increasing. Medicine makes habitable to man hitherto uninhabitable parts of the world. It prevents disease; and, with increasing theoretical and practical efficiency, medicine is learning to alleviate and cure disease and prevent injuries. Medical sciences and medical men have steadily risen in the esteem of civilized mankind. *May not the medical sciences and medical men become again the standard bearers of morality, especially of international morals?*

To accomplish these objects, it is proposed to organize as large and effective an Association as may be possible, of men and women engaged in the medical sciences or in medical practice, under the name of

THE MEDICAL BROTHERHOOD

FOR THE FURTHERANCE OF INTERNATIONAL MORALITY

It is obvious that such a Brotherhood could not exercise an important influence at once. But our modest expectation for prompt results should not prevent us from attempting *now* to take the first step in the right direction. Many important results have often had small beginnings.

A committee of physicians and medical investigators request you herewith to enroll as a member, and to declare your willingness to endorse and support the moral standard which the medical profession generally upholds when called upon to perform its patriotic duties in international strife.

It should be expressly understood that it is not the object of the proposed Brotherhood to influence the feelings and views of anyone regarding the problems involved in the present war. It is desired merely to bring to the full consciousness of the members of the medical profession the exceptional moral position which all civilized nations, even while at war, *permit* and *expect* medical men to occupy, at least so long as they remain in the medical profession and act in this capacity. This consciousness cannot fail to elevate

the moral standards of physicians. Furthermore, after the close of the present war, the Brotherhood could without doubt facilitate the reunion of the members of the medical profession of all the nations which are now at war and increase good feeling among them. A humanitarian body such as this proposed Brotherhood, if already in existence and ready for service, might and could be of the greatest usefulness in many ways.

The foregoing *Appeal*, signed by the members of the Executive and Advisory Committees, as listed above, has been widely circulated among physicians and others engaged in the advancement of medical sciences.

Any reader of this statement of the objects and proceedings of the Medical Brotherhood, who may be eligible for election to membership and who, not having enrolled as a member, desires to join the Brotherhood, is hereby invited to communicate with the President, or the Secretary, or any other officer.

*Biochemical Laboratory of Columbia University,
College of Physicians and Surgeons, New York.*

RESULTS OF STUDIES ON VITAMINES AND DEFICIENCY DISEASES, DURING THE YEARS

1913-1915*

CASIMIR FUNK

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I. INTRODUCTION

In my book on vitamins and deficiency diseases, which appeared early in 1914, and in several other articles published about the same time, these subjects were reviewed very extensively. The present article is written with the purpose of reviewing the progress of the work in this field since the publication of my book. For the benefit of any who may not be familiar with this particular branch of science, however, the chief data obtained prior to 1914 will also be briefly summarized.

The subject of vitamins owes its existence to results of the study of beriberi, a disease occurring in oriental countries where rice is used as staple food. This disease was first regarded either as an

* An abstract of this review constituted an address by the author, at the 22nd meeting of the Columbia University Biochemical Association, at the Columbia Medical School, April 9, 1915 (BIOCHEM. BULL., 1915, iv, p. 266). A review of further developments in the study of vitamins, since April, 1915, will be published by the author in a late issue of Volume V of the BIOCHEMICAL BULLETIN.

intoxication or an infection. The remarkable increase in the frequency of its incidence during the past twenty-five years suggested to several excellent workers in tropical medicine that the greater number of recent cases has been due to the introduction of modern machinery for the decortication of rice. It was shown that in certain parts of the Malay States and India, where either hand-milled rice or parboiled rice (rice steamed previous to decortication) is used, the incidence of beriberi is much less frequent than in parts where machine-milled rice is used as food. A suspicion arose that with the removal of the superficial layers of the rice grain, an ingredient is lost which is essential for the maintenance of life on a rice diet. This lost constituent was regarded, at first, as a kind of antidote, or antitoxin, against hypothetical intestinal poisons produced during the digestion of rice.

Uncertainty continued until 1897, when Eijkman observed that fowls, which had been fed on residues of food supplied in a hospital with beriberi patients, developed a disease that closely resembled human beriberi. This condition in fowls was called *Polyneuritis gallinarum*. This very important discovery paved the way for the experimental investigation of beriberi.

Eijkman found, also, that alcoholic extracts of rice-polishings cured experimental polyneuritis in chickens. He believed the effects of such extracts were due to the presence of an antidote. Schau-mann, in 1910, advanced another theory, which was based on the alleged fact that foodstuffs able to cure polyneuritis contain high percentages of organic phosphorus. He concluded, therefore, that beriberi is due to lack of organo-phosphorus compounds in the food. In accord with this view, he found that yeast is an excellent curative agent. The phosphorus-deficiency theory was advanced at a time when there was a widespread belief that lipoids had important physiological and pharmacological properties. Recently it has been shown that the animal organism is able to synthesize lipoids and other organo-phosphorus compounds from phosphoric acid, provided the remaining constituents (radicals) are available. We now know, also, that the importance hitherto attributed to lipoids was dependent very largely upon substances of basic nature which occurred incidentally in lipid fractions. The phosphorus-deficiency

theory of Schaumann had to be abandoned when it was shown, in my early experiments with yeast, that, after hydrolysis for 24 hr., with 20 percent sulfuric acid sol., yeast retained its curative properties. The experimental data indicated that the active substance was comparatively simple in chemical nature, more or less basic in character and, to some extent, thermostable in acid solution.

On the assumption that the active substance contains nitrogen, the alcoholic extracts of different foodstuffs were fractioned by means of the usual methods for the separation of organic bases. Chemical attention, however, was chiefly devoted to extracts from yeast and rice-polishings. It was found that vitamine was precipitated with phosphotungstic acid, partially with mercuric chlorid in alcoholic sol., and with silver nitrate and baryta, the latter precipitation proving to be the best for the isolation of vitamine. The curative fraction obtained in this way was very small—between 3–5 gm. from 100 k. of dry yeast, or 1000 k. of rice polishings.

This fraction, administered orally or subcutaneously to beriberi pigeons, exhibited the following effects: The animals recovered very speedily, often in 2–3 hr., but it was found impossible to keep them permanently on polished rice even when injections were repeated every few days. By further fractioning the curative material from yeast, three substances were obtained. One was definitely identified as nicotinic acid. The second substance, when completely purified, proved to be inactive but represents without doubt a new chemical substance; this is now undergoing a complete investigation. The third substance was obtained only in traces. None of these three products, given either separately or together, showed anything like the action of the original fraction. Thus far rice-polishings, as we shall see later in this résumé, have yielded nothing in this connection but nicotinic acid. It does not seem improbable that this substance is a decomposition product of an unstable vitamine.

It is not surprising that little has been achieved in the elucidation of the chemical structure of these puzzling substances. In their study unusual experimental difficulties are encountered which will be discussed below. Even a relatively simple problem like the chemical structure of adrenalin required a series of years for solution.

These preliminary results had an importance that extended beyond their application to beriberi. The substances isolated from yeast or rice-polishings, were regarded of *vital* importance, hence the name given to them, *vitamines*, a conception that found its complete justification in the number of publications, on this subject, during the last three years. Vitamines have proved to be constant constituents of the diet, equal in importance to proteins, carbohydrates, fats and salts, and not replaceable by any of these.

Plants are evidently able to synthesize vitamins. The animal organism possesses considerable synthetic capacity, as has recently been shown, but its supply of vitamins is obtained from the vegetable kingdom, a rule from which there are no known exceptions. Every animal so far investigated in this respect, when kept on an artificial but chemically definite diet, or on a purified one, exhausts its store of vitamins and gradually declines.

Beriberi is not the only disease due to dietary deficiency of vitamins. Many other diseases, such as scurvy, infantile scurvy, pellagra, rickets, sprue, several nutritional disturbances in infants, a metabolism disease in cattle called "lamziekte," etc., are due to deficiency of vitamins, and have been grouped under the name of "deficiency diseases" or *avitaminoses*. These diseases have one character in common: they are due to partial or entire deficiency of vitamins in the food. Such deficiency causes very profound changes in the functions of the central nervous system. All other symptoms, such as gastric symptoms, changes in the bones, influence on the heart and skin, etc., are only the results of the changes in the central nervous system. It is easy to realize, therefore, that these diseases are more easily prevented than cured. The changed nerves (fatty degeneration) cannot be restored to the normal condition after a certain stage of the disease is passed. This is an important point for attention in the study of "deficiency diseases."

We come now to our task of reviewing the whole subject as it developed during the years 1913 to 1915, inclusive (to date).* The distribution of the chapters accords with the arrangement in my book on vitamins, and brings the latter work up to date.

* Received for publication, May 24, 1915. The author will review, for publication in the October, 1916, issue of the *BIOCHEMICAL BULLETIN*, the further developments in vitamin research to October, 1916. [ED.]

II. BERIBERI

In spite of numerous proofs that beriberi is a "deficiency disease," a number of papers deal with the subject from a different point of view. Most of these papers have no scientific value and may be disregarded; only those will be reviewed which are based on sound observation or on experimental evidence.

Human beriberi. Now and then cases of beriberi, and even epidemics of this disease, are reported in which the point is emphasized that no polished rice was eaten. This fact is cited against the vitamine theory. We know, however, that rice as such is not the cause of the disease. White bread, sago, and, in general, any food that is naturally poor in vitamines, or is rendered deficient in them by cooking (either too prolonged or under pressure) or by extraction, is apt to cause beriberi. This point must be clearly understood.

The most prominent paper opposed to the vitamine theory of the etiology of beriberi is that of Caspari and Moszkowski (1), who consider beriberi a disease of toxic origin. They based their opinion chiefly on the results of experiments on animals. They found that addition of egg to the diet prevents the onset of the disease in animals kept on polished rice, but increase in the quantity of rice provoked the disease despite the addition of egg. They seem to believe that eggs contain an antidote for poison formed from rice, but, as we shall see in the section on the "physiology of vitamines," their results can be explained on the basis of the vitamine theory. Similar opinion was expressed by Abderhalden and Lampé (2), who found that pigeons on a diet of cooked rice developed beriberi later than those kept on raw rice. Their explanation is that during the cooking a poison was eliminated from the rice. Although the observation of these authors was confirmed in my experiments, their conclusion is quite wrong, as we shall see.

Practically all the evidence in support of the view that beriberi is a "deficiency" disease was obtained in studies on pigeons and fowls. Several authors have tried to prove that although avian beriberi (polyneuritis) is a "deficiency" disease, it has nothing in common with human beriberi. This opinion has been frequently stated, especially by Japanese authors. Thus, Shibayama (3) believes that the protective substance from rice polishings is very much less effec-

tive in human beriberi. Tasawa (4) also concludes that vitamine has no effect in human cases. Segawa (5) considers the avian disease identical with the human, but regards both as due to an intoxication. Why vitamine was found by Japanese authors to be ineffective in human cases is difficult to understand. Possibly this failure was due to selection of cases for treatment that were too advanced in their anatomical changes to be curable.

Vedder and Williams (6) prepared a vitamine-fraction from rice polishings, following my early method, and report very good results in human cases, especially of "dry" beriberi. They remark that for the wet form of beriberi, the results were not so conclusive. They assume, therefore, the existence of several vitamins, in this connection, a conception which to my mind is premature, since both forms of the disease may be very different in the severity of the symptoms.

It is interesting to note the exact composition of diets which have caused the outbreak of beriberi. Dubois and Corin (7) describe a small epidemic in the Belgian Congo, which began 4-5 months after a rice diet was instituted. The population there received a weekly addition of fresh meat to the rice diet, in a quantity which apparently was insufficient to prevent the disease. Very instructive was the outbreak of alleged beriberi on the converted cruiser "Kronprinz Wilhelm," a large number of the crew having taken the disease, although apparently they subsisted on a normal diet. A very large amount of frozen meat was available, which had been taken from captured ships from Argentine. It seems strange, at first thought, that beriberi occurred under these conditions. No cases of beriberi were observed, however, among the officers of the ship. Careful inquiry showed that the officers received daily, in addition to the ordinary food, a certain amount of fresh fruit. This latter fact classifies the disease, it seems to me, as so-called *ship-beriberi*, a disease on the borderline between beriberi and scurvy, but more like the latter, and occurs on ships where sufficient quantities of fresh provisions are not available.

Avian beriberi (Polyneuritis gallinarum). A series of papers dealt with the pathology of avian beriberi. Vedder and Clark (8) described an excellent study of *Polyneuritis gallinarum*. Schnyder (9)

reported that very little nerve degeneration occurred in birds, and that most avian cases were cured by rice-polishings, showing, in his opinion, that the paresis is not due to degenerative changes in the nerves. Segawa (5) described two distinct forms of avian beriberi; one, a simple polyneuritis; a second, more like inanition with marked aversion to a rice diet. He finds, however, that in 66 per cent of the cases both forms occur together. This investigation was conducted on fowls, some of which remained in good health for 219 days on a polished-rice diet, a result that was due, in my opinion, to the probability that the animals picked up other food than rice. The most marked pathological changes occurred in the peripheral nerves, in accord with previous findings. Intestinal catarrh, as a secondary symptom, and degeneration in the parenchymatous organs, were also observed. The same results were obtained with pigeons.

Tasawa (4) found that the symptoms of starvation in birds can be eliminated by adding egg to a polished-rice diet; in this case a picture of pure polyneuritis is obtained. He confirmed my results showing that cane sugar is able to produce beriberi in birds; that rice-polishings heated to 120° C. lose their protective power, and that potatoes exert very marked prophylactic action. Eijkman (10) claims that the disease in fowls is different from that in pigeons, and considers that only fowls develop typical polyneuritis. He injected a mixture of one part of sodium chlorid and three parts of potassium chlorid (20-40 mg.) into chickens and pigeons, and observed cures in pigeons but not in chickens. I have repeated these experiments with pigeons (Funk 11) and, as one would expect, no cures followed this treatment.

Cooper (12) has continued his studies of the amounts of food-stuffs which, in addition to polished rice, are able to prevent the onset of the disease. In addition to his previous work on the protective power of muscle, sheep-brain, fish-meat, egg-yolk, lentils and barley, he investigated the foodstuffs named in the summary on p. 311. Portions of these foodstuffs were added to the daily diet. On the basis of the results of these experiments, Cooper concluded that vitamine alone is not sufficient to induce maintenance of body weight, a conclusion that, as we shall see in the section on the "physiology of vitamines," seems to be entirely erroneous. Gib-

Foodstuffs	Amount necessary for prevention	
	Wet (natural) grams	Dry grams
Ox cerebrum	6	1.2
Ox cerebellum	12	2.4
Ox liver	3	0.9
Cow milk	> 35	> 3.5
Nuts (husked filberts)	—	2.0
Cheese	> 8	> 5.6

son (13) finds that human milk is less protective than cow milk; also, that compensated salt mixtures (often of calcium and sodium tartrate) delayed the onset of symptoms and rendered the degeneration of nerves less pronounced. Ohler (14) and Weill and Mouriquand (15) confirmed the findings of Hill and Flack (16) who found that altho white bread caused beriberi in fowls, the latter remained healthy on whole wheat bread. Ohler finds, also, that fowls remain well on whole corn but not on hominy (the inside of the corn kernel); in the latter case they develop the same condition as that on white bread, a very interesting observation to be discussed further under pellagra. Merklen (17) describes a peculiar disease in ducklings (3-4 weeks old) with symptoms of cramps and paralysis of the legs. These symptoms disappeared when the diet was variable. Possibly the disease was beriberi.

Alleged beriberi and beriberi-like diseases in other animals, especially mammals. The question whether other animals than birds and man are suitable for the study of beriberi is still open to discussion. It is undoubtedly true that no animal is able to live on polished rice or any vitamine-free food, but it seems probable that the condition described in monkeys, dogs, mice, rats, rabbits and guinea-pigs is either scurvy or a condition of general weakness. None of the conditions described (even the fatty degeneration of nerves) classifies the disease as beriberi. The symptom usually observed is a peculiar weakness of the hind legs which can hardly be interpreted as beriberi. Schnyder (9) found that the disease in mice, dogs and cats is etiologically and chemically the same as in birds, but not pathologically, for there is very little nerve degeneration. Empirically, I have divided animals into two groups: those in which the terminal purin metabolism results in allantoin; those in which uric acid is the final product. In the first group are monkeys,

dogs, cats, goats, rabbits, guinea-pigs, mice, rats and horses and cows. In these animals beriberi has not yet been produced—it occurs only in animals in which uric acid is the terminal product of purin metabolism (*e. g.*, birds and man). It is undoubtedly true that “deficiency” diseases of great practical importance occur in cattle. These diseases, which are chiefly found in cows and are called “*lamziekte*” (and perhaps “*stijfziekte*”) in South Africa, *rickets* in Australia and *Stallmangel* in Germany, resemble rickets and osteomalacia more closely than beriberi.

The problem of “*lamziekte*” is so important for South Africa that a special research laboratory was created a few years ago, under the direction of Dr. Theiler, to investigate this disease from the view point of avitaminosis. A clinical description of the disease appears in my book. A disease similar to “*lamziekte*” was described by Scheunert, Schattke and Lötsch (18), and by Lötsch (19), in horses and cattle in poor districts of the mountainous region in Saxony, where in winter only very poor and restricted diets are available for these animals. The disease called “*Stallmangel*,” which closely resembles rickets and osteomalacia, is a very serious metabolic disease, with pathological changes in the bones. The fodder fed to these animals was very deficient in calcium, magnesium and phosphorus, but Scheunert is inclined to consider that this disease is due to deficiency of vitamins

Oseki (20), in Hofmeister’s laboratory, investigated the food value of different restricted diets in mice. When put on wheat bread or on barley, a cereal frequently used in infants’ food, the mice died in 20 days, without special symptoms and without any sign of fatty degeneration in the nerves. On maize flour they lived 60 days. Whole meal rye bread was excellent food for mice, whereas oat meal, and meal prepared from peas and beans, were not satisfactory. When rye meal was extracted with water, the total extract very rapidly improved the condition of diseased mice, but the ash from the extract had only a slight beneficial effect. Extraction of rye meal with alcohol or ether had less deleterious effect on its food value than extraction with water; extraction with acetone was also without effect. By fractionation of milk, the protective substances were found in the buttermilk but not in the butter. Tachau (21),

in the same laboratory, investigated also the effects of restricted diets on mice, but from a slightly different standpoint, his experiments resembling, in certain respects, some of my own (to be described later) on the influence of additions of different food constituents. As a standard diet whole-meal bread was chosen, to which either salts, fats, or carbohydrates, were added. The mice died very soon. Addition of sodium chlorid or other salts caused very marked edema, much like this condition in infants. After adding cane sugar neither edema nor diarrhea was observed. The same applies to palmitin, but in this instance the food intake was markedly smaller. Tachau explains this phenomenon as due either to an aversion of these animals for such kinds of food, or to impaired resorptive power of the intestine. He suggests that the utilization of sugar, for instance, is dependent on the intake of proteins; in other words, that the constituents of the diet must be well balanced.

Chemistry of vitamines. RICE-POLISHINGS. Several Japanese authors have tried unsuccessfully to isolate the active substance from rice-polishings. Such attempts were described, for instance, by Murai (22) and Kondo (23). A very exhaustive study was published by Vedder and Williams (6). They repeated the fractionation of alcoholic extracts of rice-polishings, following my early method, with silver nitrate and baryta, and obtained a crystallin curative fraction. Some of the other conclusions of these authors are very interesting and confirm my statements with regard to yeast, namely that alcoholic extraction removes the vitamine only very incompletely, and that the vitamine is not very stable in the presence of fixed alkali. They found, also, that hydrolyzed extracts are very much more active than non-hydrolyzed ones.

Drummond and I (24) made a very systematic investigation of both hydrolyzed and non-hydrolyzed alcoholic extracts of rice-polishings. This work was undertaken less with the view of isolating vitamine than with the aim of separating new cleavage products of the latter. We also attempted to isolate the substances described by Suzuki, Shimamura and Odake. The latter attempt failed entirely; vitamine could not be precipitated with picric acid, and no substances were found of the type of the α - and β -acids de-

scribed by these authors. Among the substances detected with certainty were betain, nicotinic acid, cholin, guanin and adenin. Guanidin was probably also present. We also isolated a product which, in spite of several recrystallizations, had a constant melting point and was either a mixture or a very unstable compound of betain and nicotinic acid. This combination could not be isolated by crystallization but was obtained either by extraction with hot alcöhol or by precipitation with copper acetate. It was also found that the substance isolated by me from the vitamine fraction, to which the formula $C_{26}H_{20}O_9N_4$ had been ascribed, and which on analysis showed the composition of nicotinic acid, was really nicotinic acid as suggested by Barger (25).

SPINAL CORD. Voegtlin and Towles (26) prepared extracts from spinal cord and made the very interesting observation that alcoholic extract of fresh cord has less effect than that from cord which, previous to extraction, is allowed to autolyze for 2 days. One cc. of extract corresponding to 4 gm. of dry cord was enough to cure pigeons but insufficient to maintain their body weight.

YEAST. Schaumann (27) fractioned yeast with methods used for the separation of phosphatids. Yeast was extracted with 96 percent alcohol. The residue after evaporation of the alcohol was partially soluble in ether. The ethereal sol. was precipitated with acetone. The precipitate was curative. It was incomplete, however, some of the vitamine remaining in solution. The vitamine, according to Schaumann, is present in yeast in several different unstable combinations. In the same paper Schaumann describes his tests of seeds of *Phaseolus radiatus*. It was found that they lose their protective power with storage without undergoing any visible microscopic changes.

Based on my hydrolytic study of yeast, which proved that completely hydrolyzed yeast retains its curative power, Cooper (28) performed some experiments on autolysis of yeast. Fresh brewer's yeast was left for 36 hr. at 35° C. The autolyzed product was as strongly curative as the original yeast. To this mixture 95 percent alcohol was added and the liquid filtered. The filtrate was precipitated with basic lead acetate. Practically all the vitamine was found in the filtrate. The drying of yeast at 20° C., previous to autolysis,

did not diminish the curative power; and no toxic effect was observed from the administration of the autolytic products, even in ten times the curative dose.

During the past year I have continued the investigation of yeast, the chief aim having been the improvement of available methods. After many more or less unsuccessful attempts to isolate pure vitamine from such a complicated mixture as that in the alcoholic extract of yeast, it seemed desirable to devise a method that would separate the bulk of the impurities in one operation, at a stage where the vitamine is present in stable combination, and would also avoid the use of alkali, which destroys most of the vitamine. The experiments were conducted on the alcoholic extract of yeast and on autolyzed yeast.

“Alcoholic extracts.” On treating, with acetone, the phosphotungstate precipitate from alcoholic extract of yeast, about 10 percent of the total precipitate remained insoluble. The soluble fraction, which formed about 90 percent of the total, was entirely free from vitamine. The insoluble fraction, after decomposition with neutral lead acetate, was very active. The liquid, freed from excess of lead and evaporated *in vacuo*, left an entirely crystallin residue. With this preparation curative tests were performed on pigeons, with the results recorded below in the section on “physiology of vitamins.” This preparation is still a mixture of substances, however, the bulk of it being adenin. After the separation of adenin some inactive, crystallin substances were eliminated with platinic chlorid and picrolonic acid. Finally the vitamine fraction was obtained by means of mercuric chlorid in alcoholic sol.

Autolyzed yeast. As already recorded in one of my early papers, alcoholic extraction presents the disadvantage of being very incomplete, most of the vitamine remaining in the residue. On the other hand it offers the advantage that a large proportion of the impurities remain behind. Fractionations were therefore conducted with autolyzed yeast which, according to Cooper, contains the same amount of vitamine as original yeast. Here, also, the acetone-method in its original form was applied, but then another difficulty arose. The acetone-insoluble residue from the phosphotungstate precipitate amounted not to 10 percent, as in the case of the alco-

holic extract, but to 34 percent. This was a more complicated mixture. I then took advantage of an observation which was made in collaboration with Mr. Drummond. We prepared a series of phosphotungstates from several natural, chemically pure, bases: cholin, betain, nicotinic acid, stachydrin, guanin, adenin, guanidin and creatinin. The solubility of these phosphotungstates, estimated in mixtures of acetone and water of various concentrations, differed considerably, indicating that the method may be of use for their separation. Solubility did not increase, however, as one would expect, with increase in the proportion of acetone, but increased in the direction of the arrows in the following sequence:

$$25\% \rightarrow 100\% \rightarrow 50\% \rightarrow 75\%.$$

By applying these findings to the fractionation of autolyzed yeast, *i. e.*, by treating the phosphotungstates obtained with the successive concentrations of acetone mentioned above, the following *percentage* results were obtained, expressed in terms of the total precipitate:

Percentage of acetone as solvent25	100	50	75	Residue
Percentage of dissolved matter46.4	16.8	13.7	9.6	13.3

We see, then, that by applying this modified acetone-method to autolyzed yeast, the residue is only slightly greater than that for the alcohol-method. The results so far obtained indicate that most of the vitamines, even in the case of autolyzed yeast, is contained in the insoluble residue. The results are very promising since, even in the case of autolyzed yeast; 86.7 percent of the impurities can be removed in a single operation, altho the separation does not seem to be as complete as it is in the case of alcoholic extract.

Physiology of vitamines. RELATIONSHIP OF VITAMINES TO LIPOIDS. One of the chief promoters of the idea that lipoids are indispensable for life was Stepp. This is a misconception based on the observation that food extracted with alcohol is rendered inadequate for life. Although the observation was quite correct in itself, the mistake in the conclusion was due to the fact that, at that time, everything soluble in so-called lipoidal solvents was assumed to be lipid. It has been very difficult to overcome the influence of this generally accepted though erroneous idea. Lately, however, Stepp

(29) has changed his attitude; he agrees, now, that he called the life-important substance, lipid, for the sake of convenience. He has found that purified lecithin, cholesterol, kephalin, cerebrin, and phytin are not able to replace the substances extracted from food with alcohol. Food extracted with ether has full nutritive value, but not after extraction with alcohol. The important substance was also insoluble in acetone.

In a second paper Stepp (30) described further extraction experiments. He found that if food is extracted first with acetone and then with alcohol, both extracts, either separately or combined, are inactive; but a primary alcoholic extract is active. In these results he sees proof that several substances are necessary for life, one of which is vitamine. Another explanation may, however, be offered for these results, namely, that vitamine was destroyed by acetone in these particular experiments. Marshall (31) also concluded that the organo-phosphorus compounds have no more therapeutic value than the inorganic ones.

Direct proof that vitamine occurs only by accident in lipid fractions was furnished by Cooper (32), who has fully confirmed my results. He found that the vitamine of voluntary muscle can be separated from the alcoholic extract by means of ether. Ether did not precipitate the vitamine in the case of brain; acetone did. The brain phosphatides, including protagon, kephalin, cholesterol and cerebrin (phrenosin), were entirely inactive. Vitamine can be completely extracted from brain with 95 percent alcohol.

In view of all these results we can safely say that vitamine is not lipoidal in character; and if lipid products cure beriberi, it seems certain that they contain vitamine as impurity.

PROBLEM OF VITAMINES AND DEFICIENCY OF SALTS IN FOOD. A number of papers dealing with the influence of a diet poor in salts, especially of phosphorus, calcium and magnesium, are based on the wrong assumption that the authors were really working with diets deficient in inorganic but not in organic constituents. Thus, food poor in phosphorus, polished rice and sago, have frequently been chosen, and the results obtained with them regarded as being due to deficiency of phosphorus or various salts. It must be insisted, however, that if the effect of a diet poor in a particular substance is

to be studied, an artificial diet ought to be used that is "complete" in all constituents except the one whose "deficiency-influence" is to be investigated.

The workers who have been concerned, thus far, with the problem of salt-deficiency are not yet aware of the fact that, when vitamine is deficient, the balance of most inorganic constituents also becomes negative. Schaumann (27) performed metabolism experiments with maize and rice preparations on rabbits and pigeons. The nitrogen-balance was negative, and was followed immediately by a negative phosphorus-balance.

The work in connection with the need for certain salts was chiefly performed in studies of rickets, osteomalacia, and growth of young animals, and will be described in the succeeding sections. Since the publication of my book, two papers of a general character on this subject have appeared and can appropriately be reviewed here. One of these papers, by Hornemann (33), deals with the salt-content in our ordinary food, which, in the opinion of several authors, is deficient especially in calcium and iron. Hornemann was not able to confirm these statements. The other paper was by Heubner (34), who has already published some work on the importance of phosphorus in the diet. In his last paper, he apparently is more inclined to accept the importance of vitamines. He finds that deficiency of phosphorus in the diet only gradually reduces the phosphorus-content in the body. In a case of combined phosphorus- and vitamine-deficiencies the quantity of lipoids in muscle was abnormally low. The addition of phosphates decreased the amount of phosphorus in the central nervous system, but had no influence on the bones or muscles.

BERIBERI PRODUCED BY A SYNTHETIC VITAMINE-FREE DIET. I have found (35) that pigeons develop typical beriberi, on a diet consisting of casein, starch, lard, sugar and salts, as rapidly as on polished rice. The onset of the symptoms is hastened if, prior to its use in this way, the casein is purified by extraction with hot alcohol or water, as we see from the appended summary:

Uncooked diet		Cooked diet	
Onset of beriberi	Death	Onset of beriberi	Death
37 days	40 days	27 days	31 days

By using casein extracted with hot alcohol, the animals developed beriberi in 26 days, and died in 28 days; much earlier than with unpurified casein. A very convenient technic was used for these experiments. The diet was transformed by means of pill machines into pills and aliquot parts of the initial mixture were forcibly fed.

RÔLE OF VITAMINES IN METABOLISM. An investigation was suggested to me by the paper of Abderhalden and Lampé (2), who found that pigeons, on boiled rice, develop beriberi later than those on raw rice. This result was attributed by these authors to the elimination from the rice of a poison during the process of cooking. They doubted, for this reason, the existence of vitamins. For a long time I could not make out where the mistake of Abderhalden and Lampé's had occurred until I repeated their experiments (35). The mistake was at once apparent and was a very simple one. To each of two sets of pigeons I proposed to feed 30 gm. of either raw or cooked rice, daily. But it was soon found impossible to feed 30 gm. of *cooked* rice, daily, because of its enormous bulk, the weight of 30 gm. of rice after cooking being between 150 and 200 gm. This preliminary result suggested that the results of Abderhalden and Lampé were due to the use of unequal amounts of rice. The original statement of these authors was found to be correct:

Cooked rice		Raw rice	
Onset of beriberi	Death	Onset of beriberi	Death
44 days	44 days	25 days	26 days

These figures represent average results. By giving to each pigeon exactly 10 gm. of rice, cooked or raw, daily, the following results were obtained:

Cooked rice		Raw rice	
Onset of beriberi	Death	Onset of beriberi	Death
27 days	29 days	28 days	30 days

After it had been ascertained that the onset of symptoms of beriberi had some connection with the quantity of food consumed, an experiment was performed with different amounts of raw rice. The following results were obtained (36): Pigeons were fed 0.5 gm., 5 gm., 10 gm. or 20 gm., daily. The animals on 0.5 gm. a day died with starvation-symptoms, a fact which does not agree with the statement of Chamberlain, Bloombergh and Kilbourne, who claim to

have observed beriberi in starving fowls. On the contrary, it seems certain that if no food is metabolized beriberi does not occur. The results with other quantities of raw rice are recorded below:

Raw rice	5 gm.	10 gm.	20 gm.
Onset of beriberi (days)	39	36	22
Death (days)	42	38	22

These results made it necessary to ascertain which of the regular food-constituents possesses this quickening action on the onset of beriberi. This problem could be solved by varying the amounts of the different constituents in an artificial diet. Four such diets were prepared. The composition of the diet, and the results of the feeding tests, are recorded below:

Diet	Salts, gm.	Casein, gm.	Sugar, gm.	Fat, gm.	Starch, gm.	Onset of beriberi, days
A	4	60	12	12	12	30
B	4	12	12	60	12	40
C	4	12	12	12	60	24
D	4	12	60	12	12	28

From the results of this experiment the conclusion is justified that the metabolism of carbohydrates, starch particularly, requires and uses up the largest amount of available vitamine.

To complete these findings Funk and v. Schönborn (37) studied the influence of the foregoing diets, with reference to the content of glycogen in the liver and of sugar in the blood. We aimed to determine in which stage of carbohydrate metabolism vitamines play an active rôle. The results so far obtained are not very clear, but they suggest that vitamine is involved in the synthesis of glycogen in the liver. We obtained the following results for pigeons.

Normal diet: *Glycogen* (liver), 1.17%; *sugar* (blood), 0.1%.

Full artificial diet (percent—casein 12, starch 28, fat 28, sugar 28, salts 4): *Glycogen*, 0.48%; *sugar*, 0.15%.

Carbohydrate-free diet (percent—casein 12, fat 42, salts 4, made up to 100 with french chalk): *Glycogen*, 0.33%; *sugar*, 0.21%.

Starch-free diet (percent—casein 12, sugar 42, fat 42, salts 4): *Glycogen*, 0.68%; *sugar*, 0.21%.

Fat-free diet (percent—casein 12, sugar 42, starch 42, salts 4): *Glycogen*, 4.3%; *sugar*, 0.15%.

Sugar-free diet (percent—casein 12, starch 42, fat 42, salts 4) :
Glycogen, 0; *sugar*, 0.26%.

The latter diet, with addition of vitamine: *Glycogen*, 0.6%;
sugar, 0.19%.

We see, from the results of the above experiment, that, on vitamine-free diets, marked hyperglycemia developed, with partial or entire disappearance of hepatic glycogen. This result is especially marked in the case of carbohydrate-free diets, and also on starch-free diets, but is most pronounced on sugar-free diets. These results seem to suggest that, in the absence of vitamine, synthesis of glycogen from protein and fat is greatly diminished. The result on a fat-free diet further shows that the presence of fat in the diet prevents the formation of glycogen in the complete absence of vitamine. The addition of vitamine had a good effect in diminishing the hyperglycemia and increasing the glycogen-content in the liver. These studies will be extended to the action of adrenalin, phlorhizin and thyroid-extract in animals on vitamine-free diets.

Braddon and Cooper (38) confirmed my results regarding the utilization of vitamine in carbohydrate metabolism.

It was a problem of great importance to determine whether polished rice and vitamine form a "complete" diet. Although many investigators accepted vitamine as a substance that is able to prevent beriberi, some considered, however, that, for the maintenance of body-weight, other complicated phosphorus compounds are necessary. By using the fraction I obtained recently from yeast, with the acetone method, I was able (39) to cure very quickly, and to induce an actual gain in weight, on a diet of polished rice. The animals were kept in good health for a month, on repeated injections, with marked appetite for polished rice, so that the usual forced feeding was unnecessary. The experiment had to be discontinued, after that length of time, because of lack of material and also because of infections, as the vitamine solution could not be sterilized without risk of destroying its curative properties.

Cooper found (12) that vitamine, when given in the form of normal food, is not completely resorbed but a part of it appears in the feces. Schaumann (27) learned that vitamine passes into the circulation of some animals. It seems to me that this problem could

be investigated by comparing two pigeons, one fed on polished rice and the other on a normal diet. A similar experiment was performed by Morpurgo and Satta (40), who kept two mice in parabiosis for long periods, one of which was fed sucrose alone, the other a normal diet. The former received vitamine and other necessary constituents from the general blood-supply. I have shown (35) that pigeons, during attacks of beriberi, are able to utilize only a part of their bodily stock of vitamine—the part contained in the less vital tissues, *e. g.*, muscle. Beriberi-pigeons lose about 25–40 per cent of their body-weight; but, if such a pigeon is extracted with alcohol and the evaporated alcoholic extract given *per os* to another beriberi-pigeon, the latter quickly recovers. No toxic effect of these extracts was observed.

BERIBERI AND GLANDS OF INTERNAL SECRETION. The products of glands of internal secretion show, in their chemical character, a certain degree of resemblance to vitamines. Vitamines might be the precursors of these substances. It is too early to attack this problem chemically, but Douglas and I (41) compared the pathological changes produced in this relation in beriberi pigeons. We examined pituitary, thyroid, suprarenals, ovary, testes, kidney, liver, pancreas and spleen. The glands examined were diminished in size in every case; microscopically there were marked degenerative changes in the most important cells, only the framework remaining. The most marked change was the entire disappearance of the thymus, a symptom which must be regarded, however, as due more likely to inanition than to beriberi. I found later, however, that the thymus appears very quickly when vitamine is administered.

Douglas (42), at my suggestion and by using the animals from my feeding experiments, investigated the pathological changes in thyroid under the influence of various diets, particularly of diets of polished rice. No special changes were found, but it was observed that the colloid of the vesicles had a tendency to disappear. The most interesting glands of internal secretion that remain to be investigated are the parathyroids, the removal of which induces symptoms somewhat similar to those of beriberi.

III. SCURVY AND INFANTILE SCURVY (MILK PROBLEM)

Progress in the chemistry of scurvy-vitamine has naturally been very much slower than in that of beriberi-vitamine, the former substance being even less stable than the latter. Holst and Fröhlich (43) have continued their studies on the extracts of various vegetables. They found that cabbage, dried at 37° C. and kept in a desiccator, retains its curative properties for fifteen months. They also prepared an active alcoholic-glycerol extract from cabbage. Different vegetables can be successfully extracted for vitamine either with water alone or with 80 percent alcohol containing 0.5 percent of citric acid. Freudenberg (44) found that antiscorbutic substance can be extracted from various vegetables by means of alcohol, a behaviour which shows remarkable analogies with the beriberi-vitamine. This finding was confirmed by Freise (45) who prepared an active alcoholic extract from turnips.

As regards the *etiology of scurvy* it seems worth while to record the composition of the kind of diet that occasions scurvy in adults. In an editorial in the *Bulletin of Tropical Diseases* (46) there is described a diet that occasioned a number of cases of scurvy, with very marked fever, in the Burma Prison. It was found that addition of vegetables, milk, meat, or fish, had practically no influence, but the addition of sweet potatoes was effective. The composition of the daily diet was as follows:

	Oz.		Oz.
Rice (husked)	24	Condiments	0.125
Beans	4	Fish paste	0.5
Vegetables	10	Salt	0.25
Oil (vegetable)	0.5	-	-

In this connection it is also interesting to note the occurrence of several hundred cases of ship-beriberi among the sailors of the commerce-raider "Kronprinz Wilhelm," which recently arrived in a port of the United States. The sailors had a liberal diet of frozen meat but an inadequate supply of fresh vegetables and fruit. A report on the composition of the diets of sailors on the different steamship lines can be found in a paper by Markl (47).

Extensive epidemics of scurvy occurred in the mines of Southern Rhodesia, as reported by Fleming, Macaulay and Clark (48). The diet in these mines had the composition indicated on p. 324.

Mealie meal (milled maize)	2 lb. daily	Beans	2 lb. weekly
Meat	1 lb. weekly	Monkey nuts	1.5-2 lb. weekly
		Salt	ad. lib.

Here we find cases of scurvy on a diet of milled maize. The composition of milled maize will be indicated in the section on pellagra (V), with some interesting reflections upon the influence of diet on resistance to infectious diseases. Darling (49) described scurvy cases in the Rand due to overmilled maize. He finds, in these cases, hypertrophy and dilatation of the right heart, fatty degeneration of the heart muscle, severe degeneration of the vagus, which show the relationship of beriberi to scurvy

Scurvy in animals. A description of a very complete investigation of scurvy in animals appears in a book on Infantile Scurvy, by Hart and Lessing (50). They studied various animals, subject to scurvy, in which allantoin is an end-product of purin metabolism. From their account it is apparent that the most suitable animal for the study of scurvy is the monkey, the pathological lesions corresponding with those of human scurvy. In other animals the following changes were noted on different diets:

<i>Food</i>	<i>Animals</i>	<i>Lesions</i>
Oats	Rabbits	Fragility of bones
"	Guinea-pigs	Scurvy
"	Mice, rats, cats	No lesions
"	Pigs	Scurvy and beriberi
Maize	Guinea-pigs	Scurvy
Sterilized milk	Calves	Fragility of bones
"	Young rats	Arrest of growth

The last fact above will be discussed in the chapter on growth.

Infantile scurvy. The identity of infantile scurvy and adult scurvy does not require further discussion. It is also universally admitted that infantile scurvy is caused by overheated milk. Hess and Fish (51) have found that scurvy develops in infants fed on milk pasteurized at 145° F. for 30 min. The cases could be cured either with raw milk, fruit-juices or by addition to milk of potato-water. Cod liver oil proved to be inactive in cases of scurvy. The resistance of blood vessels was found to be weaker than in normal individuals. Another interesting finding by these authors was the fact that orange-peel extract was just as effective as orange juice

itself. A sample of such an extract was kindly given to me. The extract contained 0.027 gm. of nitrogen per 100 cc.—it consisted, as we see, not only of essential oils, but also of nitrogenous matter. The acidity of the extract was slight, corresponding to that of 8 cc. of *n*/10 sodium hydroxid sol. per 100 cc. of extract, with phenolphthalein the indicator.

Metabolism experiments on children with Barlow's disease were performed by Lust and Klocman (52) who found, in active stages of the disease, a positive balance of nitrogen, chlorin, ash, calcium and phosphorus; in recovery, a negative balance of the same. Bahrdt and Edelstein (53) have analyzed various organs in Barlow's disease. They found that bone marrow contained only 0.2–0.3 of the normal calcium content and 0.2–0.25 of the normal proportion of phosphorus, as in rickets; the marrow was also poor in dry substance. Muscle was poor in calcium.

It is now admitted by leading pediatricists that, in cases where highly pasteurized milk is given, additions of fruit or potato-juice as an *antiscorbuticum* is absolutely essential. It is admitted, then, that the boiling or heating of milk changes very markedly its food value. This fact has considerably changed, since the appearance of my book, the aspect of the *milk problem*, especially with regard to the accredited value of boiled milk in infant nutrition. Beriberi-vitamine is stable enough to sustain a reasonable degree of pasteurization, but not sterilization; the antiscorbutic substance can be added after pasteurization. But the question whether scurvy-vitamine is the only thermolabile substance of vital importance is still open. In her first report on the value of boiled and raw milk, Lane-Clayton (54) concluded that there was no difference in value between them. She changed her opinion, to some extent, in her second report, where she considered the question of vitamins in relation to the heating of milk.

Sittler (55) described results obtained in the milk kitchen in the Children's Clinic of Marburg University. There milk is heated for 5 min. in a water bath. No cases of scurvy were observed with such milk. A very interesting and practically unique experiment with twins is described by this author. One of the twins was breast-fed and served as a control; the other infant received the milk from

the same wet nurse—in the first period, heated to 60° C. in a water bath; in the second period, boiled for 3–4 min. At the end of the experiment the second child weighed 400 gm. less, and was weaker than the control.

On the other hand Dennett (56) found that prolonged use of boiled milk does not necessarily cause rickets. Scurvy can be avoided by adding antiscorbutica. He found that boiled milk is just as digestible as raw milk but is more liable to cause indigestion. He expressed the opinion, however, that further study of this subject was desirable. Dennett's paper was read before the American Medical Association. In the ensuing discussion, Neff said that he noted the development of scurvy on a diet of raw milk, a condition which in my opinion must be extremely rare and due, very likely, to natural deficiency of scurvy-vitamine in the particular milk involved. Lowenberg cured scurvy with raw milk; in his own cases he had not seen scurvy develop with a diet of boiled milk to which had been added meat broth and vegetable broth. Scott expressed the opinion that the boiling of milk diminishes its food value. Graves saw a case of scurvy which resulted from a diet of boiled milk and thinks additions of antiscorbutica to boiled milk are necessary to prevent scurvy under such conditions.

IV. RELATIONSHIP BETWEEN BERIBERI, SCURVY AND PELLAGRA

These diseases show to a careful observer some connecting links. Darling described scurvy that developed on a diet of overmilled maize. Ohler was able to produce beriberi with maize-meal in fowls. I was not able to produce any disturbance in pigeons, however, by feeding them on highly milled maize. Stannus (57) described 131 cases of pellagra, on a rice diet, in Central Prison, in Zomba (Nyasa-land). The diet there consisted of 1½ lb. of rice daily, with salt. Vegetables, fish or meat were given only occasionally—about once in a fortnight.

The question arises: What are the differences among diets that occasion the outbreak of these entirely different though closely allied diseases. Few words of explanation are necessary here. Beriberi occurs on diets which consist chiefly of starchy food, *e. g.*, polished rice, white bread, tapioca, sago and other vitamine-free

or vitamine-poor foods. In the latter case an increase in the quantity of cereals may induce beriberi, for the quantity of vitamine is insufficient. Small additions of meat, once a week, or vegetables, are not sufficient to prevent beriberi, but since such additions usually are eaten, no *scurvy* results. In the case of beriberi the etiology is clear—it occurs on starchy food with a negative or insufficient supply of beriberi-vitamine. We must remember that in accord with the results of my experiments on pigeons, beriberi does not occur on a maize diet.

The etiology of *scurvy* has also been entirely cleared up. The disease breaks out when dry food, in most cases such as has been stored for a long time, is eaten or when sterilized food (canned foods) is eaten. Beriberi does not occur in such cases because the beriberi-vitamine is stable enough to resist storage for a long time; but the beriberi-vitamine is incapable of preventing *scurvy*.

The pellagra cases of Stannus had pellagra and not beriberi because the rice was only partially decorticated. In my opinion these cases are very important, for they give us a clue to the etiology of pellagra. These cases suffered from pellagra, and not from beriberi and *scurvy*, because the food contained enough beriberi-vitamine to prevent the outbreak of beriberi, and there were apparently enough fresh vegetables and fruit to prevent an outbreak of acute *scurvy*.

As a general conclusion, and at the same time as a working hypothesis, I regard pellagra not as a separate disease but as a very chronic disorder due to partial insufficiency of beriberi- and *scurvy*-vitamines.

V. PELLAGRA

Pathology. The problem of pellagra, for this country and for other maize-eating countries, is one of the utmost importance. Lavinder (58) gives the number of pellagrins in the United States, between 1907 and 1912, as 19,915 with a 40 percent mortality. Of these cases, nearly 55 percent were reported in Oklahoma, Arkansas and Texas. Devoto (59) made an excellent clinical study of pellagra. He described the diet of Italian peasants in pellagra districts, which in winter consisted only of maize. Acute erythema appeared early in March. The first symptoms of the disease are weariness, lassitude, loss of weight, anorexia, paresis of the legs. In this stage

the disease can be stopped by a change of diet; and, usually, the cases improve in July and August when the diet is better.

Devoto often found alimentary glycosuria in his cases, an observation that corresponds with my results on pigeons. Bardin (60) found an increase in lymphocytes, large and small, at the expense of the polymorphonuclear neutrophiles, a fact that I have noted in experimental beriberi in pigeons. Beeson (61) found, in 25 cases of pellagra, complications with disease of the thyroid gland. In one of these cases, with enlarged thyroid, pellagra disappeared when the thyroid trouble ceased. Nicolaidi (62) found that, in acute pellagra, there are enormous losses of all kinds of nutritive constituents, mostly through the feces; losses far greater than those observed in chronic enteritis. In chronic pellagra a negative balance, chiefly through loss in the feces, was found concerning salts, phosphorus, magnesium, sodium and chlorin. Albertoni and Tullio (63) reported a negative nitrogen-balance on a maize diet, which became positive after addition of meat. Myers and Fine (64) performed metabolism experiments on pellagrins, put not on their original diet but on normal ordinary food. The utilization of various food-stuffs was very slightly lower than in the controls. There were low creatin and creatinin excretions, and a high indicanuria combined with an increase in the amount of ethereal sulfates.

Siler and Garrison (65) found the disease prevalent among women. Thus, in Spartanburg, S. C., out of 282 cases the incidence of the disease was 3 females to 1 male. It is very frequent after childbirth. Grimm (66) observed that pellagra occurred in women twice as often as in men. As predisposing causes he considers pregnancy, lactation, puerperal fever and eclampsia. Weston (67) described 15 cases of infantile pellagra in one of which the mother was pellagrous. As a remedy he recommends weaning and the maintenance of the best hygienic surroundings. The cases of infantile pellagra complete the analogy of this disease with infantile beriberi and scurvy.

Etiology. Since the appearance of my book many authors see in the diet the cause of pellagra. Weiss (68) reported cases in Rovereto, in Austrian Tyrol, where the number diminished from 8,053 in 1904 to 3,503 in 1912, as a result of improved dietary con-

ditions. The peasants there now eat polenta two to three times a day, but with additional food. They are encouraged by the authorities to keep poultry and to use garden products as food.

Perez (69) reported on the diet in the Canary Islands, which consists chiefly of *gofio*, a mixture in equal parts of wheat and maize, first roasted and then ground. This finding would be of practical importance, as no cases of pellagra occur there, provided the observations were correct, that is to say, if no fresh food was eaten, which is hardly possible.

McDonald (70) described cases of pellagra, in Antigua, that developed on diets of corn-meal and dried fish, a typical scurvy-producing combination. The disease occurs in Antigua only in blacks, although the white population eats maize as a part of a mixed diet. The author is inclined to accept the "deficiency" theory for pellagra.

In this country the food theory, if not the vitamine theory, is now generally accepted. Among the champions of this view we may count the following authors. Grimm (66) stated that most pellagrins live on corn-bread and hominy. He found the disease prevalent among paupers—in 258 cases out of 323, and chiefly among whites. Goldberger (71) found that attendants, nurses, and doctors, in pellagra asylums, never suffer from the disease because they receive an adequate diet. He advocated fresh meat, eggs and milk instead of the cereals and canned meat, so largely consumed in Southern States. Siler, Garrison and MacNeal (72) described 847 cases in Spartanburg county, S. C. They do not endorse the "deficiency" theory, but they admit that the result of dietary treatment was good. They state, however, that the patients relapsed when returned to their original environment. These authors should not have been surprised at this outcome, for the patients probably returned, also, to their previous imperfect diets. It is interesting here to note that, as early as 1835, Rayer (73) expressed the view that pellagrins should change their habits and occupation, which, in his opinion, alleviated the symptoms of the disease.

Finally, I wish to quote the opinion of Voegtlin (74), who admits the importance of the diet as an etiological factor and considers the following three possibilities:

1. A chronic intoxication by soluble aluminium compounds;
2. Lack of vitamines (Casimir Funk);
3. Lack of certain amino-acids.

Treatment. In some parts of Italy the government initiated very energetic measures against pellagra. Alpago-Novello (75) has summarized the work undertaken in the province of Belluno, where pellagra diminished very rapidly when the cultivation of beet-root and potatoes was substituted for that of maize. The pellagra commission of the district of Mariano, near Triest, has distributed, among the population, seeds of sweet potato (76). In Southern Tyrol Kleiminger (77) cured 13 cases of lunacy, on the basis of pellagra, by a change of diet even without addition of fresh vegetables and meat. Allison (78), Sylvester (79), and Elebash (80) recommend fruit or fruit-juices, milk, eggs, and vegetables. Lorenz (81) treated 27 cases of pellagra with what he calls an excessive diet for eight weeks, with the following result: 7 cases died, 3 remained stationary, 13 cases improved, and 4 recovered completely, even in respect to the mental symptoms. Quite recently Blosser (82) observed 133 cases of pellagra, all of which, except 3, ate cane products freely. Exclusion of all partially refined sugars and sirups was followed by a cure in 121 cases and improvement in 8 cases; 4 died. It would be interesting to know the percentage of cane products in the original food of these patients. If they represented the bulk of the nutritive substances, the results of this experiment would bear striking analogy to those of my experiments on pigeons, in which beriberi was produced by a diet of sugar alone.

As a practical conclusion to this section on pellagra, I wish to draw attention to the following point. Pellagra is, beyond doubt, a disease peculiar to districts inhabited by poor people, *i. e.*, in districts where the population lacks the financial means to supplement the routine diet with imported foods. We know of many examples that illustrate the limitation of pellagra to areas devoted to maize-culture. The peasants of Poland and Russia live exclusively on soup consisting of potato and cabbage, and whole-rye bread. One could not imagine a cheaper and less varying food. We can safely state, however, that deficiency diseases are practically unknown in these countries, with the sole exception that during periods of bad harvests, scurvy epidemics sometimes appear. As, however, we

approach the zone where the cultivation of potato ceases and maize plantations appear, as in South Russia, on the Roumanian border, or in Western Galicia, we encounter cases of pellagra. It seems, therefore, that the United States government could eradicate pellagra entirely by an introduction of potato culture in pellagra districts, as I have already suggested. It is obvious that such plans could not be executed at once but it seems advisable that such an experiment should be tried on a large scale.

We may now consider another point of practical importance, namely, the question of the preparation or milling of maize, previous to its use as a food.

Milling of maize. On studying the data for the mortality from pellagra in different countries I was surprised to note the discrepancies among the figures. Thus, for example, in Italy and Egypt the mortality is very low, attaining only 4 percent, whereas in the United States the mortality is as high as 20-25 percent. The differences in mortality may be due to several causes as, for example, more or less exclusive diet of maize. But among the possibilities one factor requires particular attention, *i. e.*, the question of the preparation of maize. My attention was drawn to this point by Dr. Macaulay, of Cape Colony, who was one of the members of the commission for the investigation of scurvy in the mines. He noticed that the maize used there as a staple food undergoes a severe milling process, during which 14 percent of the grain is lost for human use and employed for feeding cattle. The population there suffered severely from scurvy, as has already been stated. Macaulay tried to introduce legislative measures prohibiting such excessive milling but encountered considerable opposition from the farmers, who had, in the wastage of the milling process, a very cheap and nourishing food for cattle. Finally, however, he succeeded in placing on the market maize which had been subjected to less extensive milling and which represented 97 percent of the total grain. Samples of this maize sent to me were analyzed. The results are given in the table (84) on p. 332.

We see, from the accompanying diagram, that the chief nutritive substances are localized, in the South African maize, in the aleurone layer of the kernel near the surface of the grain, and in the germ.

Data pertaining to milled maize (97 percent of the total grain). See page 331.

	Water	Ash	Nitrogen				Phosphorus: P_2O_5	Fat	Fatty acids	Cholesterol	Lipoid- P_2O_5	Color-reaction: Folin and Macallum	
			Kjeldahl	Dumas	Melanin	Van Slyke						Alcoholic extract	
												Hot	Cold
1. Whole maize grain.	12.71	1.56	1.73	1.74	0.14	0.99	0.56	4.5	3.88	0.247	0.0154	0.35	0.55
2. Highly milled meal, 86%	12.63	1.48	1.67	1.73	0.13	0.95	0.36	3.87	3.36	0.22	0.0098	0.23	0.45
3. First milling from No. 2	10.48	2.09	1.23	—	0.14	0.61	0.30	4.66	4.18	0.357	0.0164	1.00	1.00
4. Second milling from No. 2	10.71	4.04	2.31	2.34	0.16	1.40	1.43	12.79	11.09	0.438	0.0353	0.70	0.60
5. Slightly milled meal, 97%	12.41	1.60	1.73	1.84	0.12	1.00	0.54	4.21	3.63	0.233	0.0153	0.30	0.45
9. Bran from 5, above	10.55	1.40	0.65	—	0.07	0.30	0.23	1.86	1.58	0.271	0.0084	0.35	0.70

During the process of milling, a large part of these layers is milled away, as is shown by the figures in the above table.

Pigeons were fed this highly milled maize for several weeks, but they remained in perfect health and differed totally in their be-

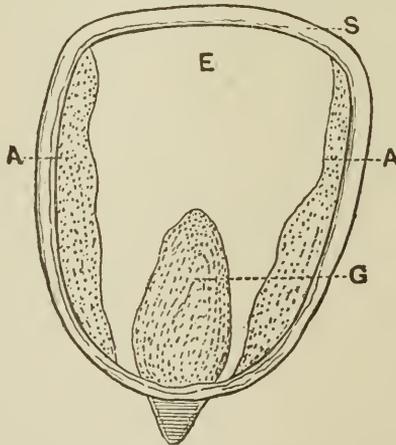


DIAGRAM OF A SECTION OF A MAIZE GRAIN

S: Skin. E: Endosperm. A: Aleurone layers. G: Germ

havior from fowls which, according to Ohler, develop, on such a diet, a disease that he considered identical with beriberi. Driscoll (85) observed that chickens, fed on maize with the outer layer removed, developed erythema on the legs, and acquired a disease simi-

lar to human pellagra. The symptoms disappeared in three weeks, when the chickens were fed on whole corn. In four cases of pellagra in man the same author saw good results on a diet of cornmeal to which bran had been added. The work of Driscoll requires confirmation, before his conclusions can be accepted; but the problem of the influence of the mode of milling of maize on the incidence and severity of the symptoms can not be disregarded, especially in view of the observations of Macaulay and Darling on the occurrence of scurvy on overmilled maize.

Analyses of various samples of maize have been made by Juritz (86) and MacCrae (87), the latter having determined especially the phosphorus-content in maize milled to different degrees, and confirmed fully my results. Poppe (88), who described the diet of the working class in Belgian Congo (1 k. of maize meal, 1.4 k. of corn, 0.14 k. of meat and 14 gm. of salt, daily), stated that the water from cooked maize should not be thrown away, for 36.2 percent of the nutritive ingredients would thus be rejected (89).

Weiss (90) found that pellagra is prevalent, in Tyrol, in districts where fine maize, without the husk, is used as food. Nightingale (91) gives a similar account but of much greater value, as his conclusions are based on 1210 cases. He described the diet in Southern Rhodesia, where mealie meal is passed through a sieve that retains the husk. The husk proved to be an excellent food for cows, increasing greatly the yield of milk. This maize preparation caused a disease he called zeism, which is nothing but pellagra of a mild type. On the other hand, the hand-milled maize (rapoko) proved to be preventive and curative. Cod-liver oil was also of great value, in his hands, for the treatment of pellagra.

In summarizing the results obtained and the views expressed by various authors, it seems to me that we already possess all the knowledge necessary successfully to combat pellagra.

VI. SPRUE

Our knowledge of sprue advances with our knowledge of pellagra. This disease occurs less frequently than pellagra, however, and less work has been published on the subject. The disease results directly from deficient diets. Cantlie (92), who has done

much work on this disease, continues to regard sprue as due to infection, but the data from his own paper indicate that it is dietary in origin. He saw, in many sprue cases, tetany with anemia, often accompanied by unconsciousness and fever up to 103° F. Continued fever indicates the last and fatal stages, results (very likely) of secondary infection. For treatment he recommends strawberries, ripe raw gooseberries and the juice of blackberries. Bahr (93) found that the disease is more common in women (analogy to pellagra). His post mortem findings were: great waste of internal organs and inflamed intestines. Leede (94) also recommended strawberries as a cure; but he made an interesting observation from our point of view, namely, that *preserved* strawberries are not equal in curative power to *fresh* fruit. He suggested that the beneficial principle is destroyed by heat. Mühlens (95) believes that sprue is sometimes complicated by dysentery, when, besides ingestion of strawberries, the administration of emetine is indicated. He is inclined to accept the deficiency theory. Werner (96) observed scurvy symptoms (petechiæ) in a case of sprue.

VII. RICKETS

Our knowledge of rickets has not increased sufficiently, in this connection, to advance us beyond the stage of the very promising hypothesis that the disease is due to deficiency of vitamines. Advance has been slow because there have been many cases where the differential diagnosis of rickets and bone porosity was impossible. Bone porosity (*Fragilitas ossium*) is not improved by treatment with calcium salts, phosphorus or cod-liver oil, according to Ostheimer (97), but by good food. Ostheimer considers this disease a result of faulty metabolism during pregnancy (congenital fault of metabolism) and that it has nothing to do with rickets or osteomalacia. On the other hand we have to differentiate, clinically, between cases that improve on dietary additions of calcium and phosphorus only, and those that do not improve after the further addition of vitamines to the diet. The latter types of cases must not be regarded as rickets.

As we have seen in the preceding chapters, special attention must be given to the fact that, in experiments with so called calcium- and

phosphorus-poor diets, the latter are usually also deficient in vitamins. This fact applies, for instance, to the work of Schmorl (98) who investigated effects in puppies fed with Heubner's diet. Schmorl admits, however, a possible deficiency of vitamins in this diet. He found diminished formation of bone, a decreased number of osteoblasts and an increased amount of osteoclasts. The bones were very soft despite a sufficient supply of calcium salts in the food. The lesions in the bones resembled those of Barlow's disease; but there were small hemorrhages in the sub-cartilaginous zones, as in human rickets. Schmorl sees a possible connection between a deficiency of vitamins and a disturbance of internal secretion. Weiss (99) treated ten rickety children with hypophysochrom of Klotz and obtained a favorable impression as the result.

Investigators who believe there is a definite connection between rickets and disturbance of internal secretion meet the truth half way, as it is possible that certain vitamins, which are necessary for the prevention of this disease, may be used in the organism as "starting material" for the activity of the glands of internal secretion, and in this way may play a rôle in the prevention of rickets. We may accordingly disregard the results obtained by Rominger (100) with the aid of Abderhalden's dialysis method, which led him to conclude that there is no evidence of a disturbance of internal secretions in rickets.

As to the cause of rickets, very few data have been obtained recently. Sittler (55) saw cases of rickets on sterilized commercial butter-milk, to which starch in the form of cereals had been added. He was able to cure these cases with raw milk. More work was done on the therapeutic action of cod-liver oil, either as such or in conjunction with phosphorus. Schloss (101) described the effect of cod-liver oil with phosphorus on three breast-fed rickety children and found negative calcium- and phosphorus-balances. An addition of calcium acetate rendered the calcium-balance positive. He believed there was a definite connection between the calcium- and magnesium-balance, which showed opposite tendencies, one being positive when the other was negative, and vice versa. Frank and Schloss (102) could not find a difference between cod-liver oil alone, or in combination with phosphorus, as regards effects on protein

metabolism. It seems that the phosphorus could be dropped from the combination without loss of advantage.

Regarding the influence of cod-liver oil on calcium metabolism, we find in the literature opinions that disagree with those of Schloss, as in the case of Kurt Meyer (103), for example. In *Osteogenesis imperfecta* (congenital porosity of bones) good results with cod-liver oil were observed by Borkman (104), and with cod-liver oil and hypophysochrom by Schabad (105). The discordant results of various authors can be explained, perhaps, as we shall see later, by the use of dissimilar preparations.

The most important objection any one has advanced against the vitamine-deficiency theory of the origin of rickets is the relative frequency in the occurrence of rickets in breast-fed infants. This fact seems to me to be one of the best arguments in favor of this theory, as is indicated in the succeeding section.

VIII. CHEMISTRY OF COD-LIVER OIL

Exceedingly little work has been done on this subject. In 1888, Gautier and Mourgues (106) isolated, by extraction and by distillation at a high temperature, two nitrogenous bases called by them *morruin* and *aselin*. Judging from the character of the methods used, these substances may be regarded as secondary products resulting from the distillation, or as decomposition products of some more complicated substances. A series of papers on this subject were published by Iscovesco (107), who, working with the usual methods for the preparation of phosphatids, isolated a lipid either from cod-liver oil or from the liver of codfish. He claimed to have obtained, with this preparation, a specific action on the growth of rabbits and guinea-pigs, which could not be duplicated with any other lipid. We shall consider, in the section on growth, the effects claimed for this preparation.

During the year 1914 I took up the subject. As a basis for my investigation I relied upon the observation, of several specialists in children diseases, that more pronounced therapeutic effects have been obtained with *crude* cod-liver oil than with the highly refined pharmaceutic preparations. My experiments were made on young chickens which, when kept under laboratory conditions, develop a

disease that shows in every respect the greatest analogy if not identity with rickets in children. One observes in these animals every kind of deformation of various bones, chiefly the sternum, deformities which may proceed so far that the intake of food is rendered impossible. The mortality of these animals was very high, sometimes as high as 80 percent. (I have observed several cases of typical rickets in young growing *rats* which had been kept on artificial diets.) I tried to diminish mortality in the chickens by changing the food, by adding yeast, fresh grass and green vegetables, but only with very slight effect. It seems, therefore, that young chickens can be raised without rickets only when they are kept in the open air where they have opportunity to pick up worms.

Two samples of cod-liver oil were at my disposal: a slightly refined oil of brown color and a crude oil. By adding the two kinds of oil to the ordinary diet a marked difference was observed, the crude oil being very much more efficient in preventing the rickety condition and diminishing the mortality of the chickens. Extraction of these two samples proved that the crude oil contained a much higher proportion of nitrogenous extractives than the slightly purified oil. As my paper on this subject appears in this issue of the *BIOCHEM. BULL.* (p. 365), I shall refrain from mentioning here the details of the work (108), except to say that the extraction products will be tested clinically, as there is no reason to suppose, from our present knowledge, that there is any particular value in the administration of the oil as such.

In studying the effects of these oils in infantile rickets, or even in experimental rickets, we encounter great difficulties. In clinical cases signs of rickets persist after the actual disturbance due to a deficiency of vitamine has ceased. Marked effect of our preparation could not be expected in such cases. Even in florid cases of rickets, observation had to be extended over long periods because effects could not be expected to occur as promptly as they do after the administration of beriberi- or scurvy-vitamine.

In concluding this section I wish to express the belief that current differences of opinion regarding the therapeutic action of cod-liver oil are due to the use of highly refined oils by the clinicians.

IX. OSTEOMALACIA, SPASMOPHILIA, ECLAMPSIA

Osteomalacia may be considered as a rickety condition in adults. This disease affects only women in pregnancy and puerperium. A slight degree of this affection occurs so frequently that we see, in von Noorden's book on the Pathology of Metabolism, an expression of the view that there may be a "physiological" osteomalacia (109) in pregnant women. I have already referred to an analogous affection in cattle, called "Stallmangel." Antoine (110) described typical osteomalacia in dogs. In this review I have occasionally pointed out the frequency in the occurrence of deficiency of vitamins during pregnancy and lactation. Let me remind the reader of the occurrence of infantile scurvy and beriberi in breast-fed infants where the symptoms of the diseases may or may not be shown by the mothers. In the latter cases the mothers possess just enough vitamin to keep them free from these diseases but there is not enough vitamin in their milk to protect their infants. In the same way the infantile form of pellagra can be explained and also the occurrence of rickets in breast-fed babies.

The occurrence of rickets in breast-fed infants is often considered an argument against the vitamin-deficiency theory of the etiology of rickets. These cases occur chiefly among poor people, and the food of the mothers should undergo careful inquiry. But even among wealthy people a deficiency of vitamins is not impossible in pregnancy and lactation, for the appetite in such cases is very often not up to the demand considering the condition of the organism. Study of the nutrition in pregnancy suggests, so far as vitamins are concerned, that the quantity of these substances which would suffice under ordinary physiological conditions is often inadequate in pregnancy and lactation.

I went into this field with special interest in the mysterious etiology of *eclampsia*. Every available theory of its origin was tested but without the slightest success. This condition occurs in the late stages of pregnancy and also in the puerperium. I wish to draw the attention of the clinicians to the possibility of a deficiency of vitamins in this condition and to suggest research in this direction. We shall better understand this possibility if we compare eclampsia with beriberi, especially the spastic, tetanic form.

Very similar symptoms occur in *spasmophilia* in children and also in a condition which the German authors call "*Mehlnährschaden*." Spasmophilia occurs in children fed on large proportions of farinaceous foods and can be safely regarded as infantile beriberi. Takasu (111), for instance, describes spasmophilic dyspepsia in breast-fed babies in Japan as beriberi. Liefmann (112) was able to detect, in normal children between 10 days and 2¼ years of age, a daily excretion of 1-5 mg. of acetone. In a spasmophilic attack this excretion increases up to 93 mg. This observation, and also the fact that spasmophilic children show increased irritability of the muscles to the electric current, can be used successfully for the diagnosis of this condition and also of other avitaminoses in children.

Freudenberg and Klocman (113) recommend the use of oxy-cod-liver oil (oil treated with hydrogen peroxide) for the treatment of spasmophilia. It seems, however, that treatment with the original oil, or a concentrated extract as described under rickets, is more promising. Brüning (114) described a condition in new born white rats, when separated from their mother and fed artificially on a diet rich in carbohydrates, which he considers identical with "*Mehlnährschaden*." No pathological changes in the bones were found.

In summarizing this section I conclude that it seems highly desirable to apply the vitamine therapy to osteomalacia, spasmophilia, *Mehlnährschaden* and eclampsia. We understand in this connection chiefly the therapeutic use of yeast preparations and cod-liver oil, the latter in accord with the statements in the previous sections. If these diseases are rightly regarded by me as avitaminoses, the application of this therapy not only would bring about practically instantaneous cures but also would serve very well for the purpose of testing quickly the value of the clinical diagnosis.

X. CHEMISTRY AND PHYSIOLOGY OF GROWTH

This subject has received special attention during the past two years but no definite conclusion has as yet been attained. At present there are several theories on the chemical nature of the substance that promotes growth in young animals. The most important of

them relate to (a) Inorganic salts, especially of phosphorus; (b) lipoids, or in reality phosphatids; (c) fats or oils of peculiar constitution; (d) certain amino-acids and (e) vitamins. Which of these theories has the greatest justification the reader will be able to judge for himself from the succeeding discussion.

Theory of inorganic salts. Masslow (115), in his experiments on the growth of young dogs, studied the influence of what he thought was a diet poor in phosphorus. The diet he used had the following composition: Rice, 100 parts; egg albumen, 50 parts, with addition of potassium, sodium and calcium; sugar, 40 parts, with addition of magnesium and iron; starch, 50 parts. The dogs remained in good condition for a month, on this diet, then a great wastage of flesh, loss of appetite and death resulted. This diet was typical vitamine-free food; and the addition of phosphates and glycerophosphates had no effect, whereas the administration of "Lecithine Merck" resulted only in temporary improvement. In spite of these results Masslow regards these failures as due to lack of phosphorus.

In a second paper, on the same subject, Masslow (115) gave the results of analysis of the organs of the dogs kept on the above-mentioned diet. There was diminished content of phosphorus and ferments. Masslow (116) found that the bones underwent marked changes, with small hemorrhages resembling those of infantile scurvy. Durlach (117) carried out, on a similar diet, some experiments on dogs, but the animals did not grow on vitamine-free food.

Röhmnn (118) fed mice on artificial diets. The duration of the experiments was extended to the second generation. In the second generation, however, the diet did not suffice. Although Röhmnn concluded that an artificial diet does not have the value of ordinary food, he refused to accept the existence of any unknown chemical factors. The composition of the diets in his experiments were the following:

<i>First</i>	<i>Second</i>
12 gm. casein	14 gm. casein
4 gm. chicken protein	4 gm. chicken protein
4 gm. nucleoprotein from liver	4 gm. vitellin
180 gm. potato starch	60 gm. potato starch
	120 gm. wheat starch
12 gm. margarine	19 gm. margarine
4 gm. salt mixture	4 gm. salt mixture

The salt mixture contained 10 gm. of calcium phosphate, 40 gm. of potassium bi-phosphate, 20 gm. of sodium chlorid, 15 gm. of sodium citrate, 8 gm. of magnesium citrate, and 8 gm. of calcium lactate. This diet was inadequate for mice; they grew less rapidly than on a diet of bread and milk. The substitution of vitellin for the nucleoprotein did not yield better results. No pathological alterations were seen in these animals and, on changing this diet to a normal one, quick recoveries followed. The second diet gave good results in old mice, but in young ones no growth was observed. The substitution of a part of the vitellin in the second diet by chicken protein, or edestin, failed to affect growth. The same was true of meat-extract and meat-powder. On the other hand, addition of malt-extract or nucleoprotein from liver stimulated growth. Egg-yolk fat had an unfavorable effect on adult mice. In young mice egg-yolk prolonged life but did not stimulate growth. Very good results were also obtained when the food was mixed with yeast and baked; lecithin was without effect.

Hart and McCollum (119) performed some experiments on swine. No growth was observed either on wheat alone, or on corn meal and gluten feed. Growth occurred, however, when to the above mentioned food, potassium phosphate, potassium citrate and calcium lactate were added. Under these conditions the growth on corn meal was more successful.

Wheeler (120), in Mendel's laboratory, extended to mice, the rat studies of Osborne and Mendel, and found that mice are also well adapted for experiments on growth. Wheeler's work was undertaken at a time when Osborne and Mendel believed in a special relation between milk-salts and growth, but the results indicated the necessity of adding organic milk food, even in larger relative quantity than for rats, to induce complete growth, in which case mice grew more rapidly than rats. Osborne and Mendel (121) have amended their statement regarding the beneficial effect of protein-free milk, and of artificial protein-milk, on the growth of rats.

Hart and McCollum (122), in continuation of their studies on growth in swine, concluded that even after an addition of salts to corn- or wheat-feed only partial growth can be obtained, the pigs showing signs of paralysis with ultimate decline.

As a conclusion from these experiments we notice the complete failure to demonstrate any special value of inorganic salts in the process of growth; that even the addition of mixtures of salts to artificial diets was unable to replace the important constituents of natural diets.

Value of lipoids and phosphatids in growth. Iscovesco (107) performed some experiments on growing animals with lipoid isolated by him from cod-liver oil or from the liver of codfish. This lipoid could not be replaced in its action by any other lipoid. For instance, for young rabbits he obtained the following figures for increase in weight: Controls, 33 percent; cod-liver oil, 55 percent; the same, without the lipoid, 37 percent; olive oil, 33 percent; olive oil, with the lipoid, 56 percent.

Desani (123) fed white mice with starch and casein that had been extracted with alcohol and ether. This extraction was undertaken in order to get a food free from cholesterol. The animals died after 18-19 days with a loss of weight of 41 percent.

McCullum and Davis (124) investigated the value of lipoids from boiled eggs. The ether- and petroleum-extracts were effective growth stimulants, the acetone extract only to a slight extent.

Lander (125), who worked with a carefully purified diet, found that rats live on such a diet about 14 days, the addition of cholesterol, cholesterol esters and lecithin having no effect on growth.

MacArthur and Luckett (126) proved that lecithin, cephalin, cerebrosides and cholesterol are not vital dietary constituents for mice; but they state that a fraction of egg-yolk insoluble in ether and soluble in alcohol, probably vitamine (thermolabile), is necessary to make a complete food.

The above mentioned papers disprove the theory that lipoids are necessary for the growth and maintenance of animals.

Importance of peculiar fats and oils for growth. A new impulse in the study of the growth problem was given by the very important discovery of McCullum and Davis (127) that butter-fat or fat from egg-yolk is able to stimulate growth of rats which have declined on artificial diets. This observation was confirmed by Osborne and Mendel (128) and extended by them (129). They separated butter by centrifugation into three fractions, solid detritus,

fat fraction and butter-milk. The fat fraction which, according to these authors, contained no nitrogen and phosphorus had apparently the same effect on growing rats as the original butter; rats which were in a bad nutritive condition quickly recovered. Especially, an infectious disease of the eyes was promptly cured. The belief that this butter fraction contained no nitrogen, was used as an argument against the vitamine-theory of growth, and induced me to investigate the butter problem more closely.

Macallum and I (130) fractioned butter, by the method of Osborne and Mendel but further purified the butter-fat fraction in the following way. The fat was dissolved in acetone and shaken with a weak solution of hydrochloric acid. From 12 k. of butter 23.4 mg. of nitrogen were obtained by the Dumas method, and slightly less by the Kjeldahl method. The butter-fat, after its subjection to this process of extraction, was hydrolyzed with weak acid and again 22 mg. of nitrogen were obtained. These figures are certainly low and would suggest that the butter-fat was practically nitrogen-free, if there were not the possibility that some of the contained nitrogenous substances were volatilized during concentration *in vacuo*. This possibility must be tested.

McCollum and Davis (131) also questioned the absence of nitrogen and phosphorus from Osborne and Mendel's butter-fat. Osborne and Wakeman (132) again tested the purity of their butter preparation and found only traces of nitrogen and phosphorus.

Aron (133) has also observed beneficial effects of butter on growth. Osborne and Mendel (134) have found that old stunted rats resumed growth when the inadequate diet was changed to a "complete" one.

The possibility of a stimulation of growth with nitrogen-free butter seems to me so highly improbable that I am unable to accept the statements to that effect without further proofs. Macallum and I performed some comparative experiments with crude butter and purified butter, following our method on rats. The percentage composition of the diet was as follows: Casein, 20; starch, 41; sugar (cane), 21; butter crude or purified, 12.4; salts, 2.6. The casein was extracted for several days with boiling alcohol. Lactose was avoided as it contains a small quantity of nitrogen—according to

McCollum and Davis (135), 0.02-0.034 percent. Salts were used in the same kinds and proportions as those for the experiments by Osborne and Mendel, but no protein-free milk was added.

We did not succeed in keeping our rats longer than two months on this diet. We were unable to see any good effects of the addition of the butter. Slightly better results were obtained, however, with *crude* butter. Male rats increased in weight, after 42 days, 54 percent; females, 41 percent. At that time, in the purified butter series, several rats had already died. After 24 days the males in this group increased their weight by 45 percent, the females by 31 percent; whereas, at the same time (24 days) in the *crude* butter experiments, the weights of the males increased by 66 percent, of the females by 48 percent.

Both butter preparations were fed in an artificial diet to pigeons, the latter developing beriberi in the usual time in both series. In neither butter samples could any appreciable amount of beriberi-vitamine be detected by this method.

I would not call our experiments entirely conclusive, especially as further studies are in progress, but I received the impression that the remarkable results obtained by Osborne and Mendel are due, to a great extent, to the use of protein-free milk. It seems to me that in the experiments of Osborne and Mendel the excellent results are due to the addition of vitamine with casein, lactose, protein-free milk and butter. One of the last papers of McCollum and Davis (135) gives a less enthusiastic account of the influence of butter on growth. They believe that even the butter diet lacks something essential which is present in natural food for rats.

The study on fats was extended to cod-liver oil (Iscovesco, 107) by Osborne and Mendel (136), which was found to have the same action as butter, whereas almond oil and tri-glycerides of the fatty acids had no effect. McCollum and Davis (135) found lard, olive oil, tallow and cotton-seed oil inactive, but the ether extract of dried, ripe cod testicles and pig kidney were very active.

Recently Osborne and Mendel (137) have fractioned beef- and butter-fat more extensively. Abdominal fat of cattle and butter were dissolved in alcohol at 40° C. and allowed to crystallize. The crystallin fraction was ineffective. The oily part, which was

greater in quantity in the case of butter, was very effective. This fact strengthens my belief that the stimulating action of butter-fat may be due to vitamine. It is regrettable that Osborne and Mendel did not determine whether the oily fraction contained nitrogen.

On summarizing this section we see that the growth-promoting substance was found only in materials from sources which are known to contain vitamins, *e. g.*, milk, cod-liver oil, beef-fat, etc.

Question of thermo-stability of the growth-promoting substance. Here we also find great divergence of opinion as in the case of beriberi-vitamine. Thus, Hart and Lessing (50) observed complete arrest of growth in young rats fed on sterilized milk. On the other hand McCollum and Davis (124) were able to obtain an active extract from boiled eggs. They went even further: butter-fat was hydrolyzed (131) in petroleum ether, at room temperature, with an alcoholic potassium hydroxid solution. After neutralization the soaps were extracted with olive-oil and the growth-promoting factor was found intact. Osborne and Mendel (136) also consider the growth-promoting substance thermo-stable.

Rôle of amino-acids in growth. This problem was studied extensively by Osborne and Mendel, and by McCollum. The former authors usually arranged their experiments as they did those mentioned above. In the artificial diet, containing butter-fat and protein-free milk, the casein was replaced by protein known to be deficient or poor in certain amino-acids. It was found by Osborne and Mendel (138) that gliadin without addition of lysin was inadequate for growth. The same applies to zein without addition of tryptophan. In another paper (139) they stated that the addition of cystin and lysin made an inadequate supply of casein suitable for growth. Lactalbumin was found to promote growth very strikingly. This protein was found to yield more tryptophan than that from any other protein and they attribute the influence on growth to the presence of this amino-acid. In this inference there is the fallacy, however, that lactalbumin may contain vitamine from milk. These authors found, also, that a deficiency in any particular ingredient of the diet did not induce a corresponding compensatory increase in the food intake (140).

McCollum (141) found that the proteins of milk are superior to

any other proteins so far as growth is concerned. Street (142) has compared the value of sanato-gen and casein for growing rats but could not detect a difference between these two food-stuffs. Hektoen (143) found that the production of anti-bodies was normal in rats fed with pure vegetable proteins (Osborne and Mendel's diet).

The experiments on the influence of isolated proteins, with additions of certain amino-acids, show what had previously been demonstrated: that amino-acids, like tryptophan, tyrosin, lysin and cystin, are indispensable components of a complete diet. In experiments on growth it is obviously essential that the protein in the diet should yield all the necessary amino-acids in adequate amounts.

Importance of vitamins for growth. Until recently the growth problem had been studied only on rats, mice, dogs and pigs. I have extended these studies to chickens and, to my surprise, found that practically all the preparations which stimulated growth in rats failed to do so in young chickens. It was also found that polished rice as well as unpolished rice was entirely inadequate to stimulate growth in these animals. The best results (144) were obtained by adding yeast to an unpolished-rice diet. On these diets, however, all the chickens died in several weeks. On adding cod-liver oil to an unpolished-rice diet the animals remained in fairly good health for several months but no growth resulted (130). The addition of tumor-tissue (Rous's sarcoma) also had a stimulating effect.

In another series of experiments I tried the effect, on the growth of chickens, of an addition of phosphotungstic precipitate, and of phosphotungstic filtrate, to an unpolished-rice diet. No growth was obtained (145). It seems, therefore, that growth in chickens is dependent on such substances as are contained in living worms, for even additions of fresh grass and salad to the inadequate diets were without effect.

Experiments were also carried out on young chickens with both germinated and ungerminated oats, rice and barley, but these diets proved to be unsuitable for young chickens. The experiments will be repeated on chickens and rats.

In conjunction with Macallum (130) experiments were performed on rats in the diets for which various portions of the starch were replaced with unpolished rice or with polished rice. The per-

centage composition of each of the diets is indicated in the appended summary :

	Diet I	Diet II	Diet III	Diet IV
Casein.....	22	22	22	22
Starch.....	37	29	20.5	29
Unpolished rice.....	4	12	20.5	—
Polished rice.....	—	—	—	12
Sugar.....	21	21	21	21
Lard.....	12.4	12.4	12.4	12.4
Salt mixture.....	2.6	2.6	2.6	2.6

The experiment with polished rice terminated very early—in 29 days, all the animals dying without any sign of growth. The unpolished-rice experiment continued for 61 days. The best results were obtained with Diet III, which contained the largest amount of unpolished rice. If we take, for convenience of comparison, a period of 28 days we find the following average figures in the records of these experiments. On Diet I the rats show a weight-loss of 3 percent; on Diet II, there was a gain of 7 percent; on Diet III, a gain of 16 percent. The *chief* chemical difference between polished rice and unpolished rice, so far as we know, is the difference in the proportion of vitamine. These results suggest very strongly that vitamine plays an important if not a decisive rôle in the experiments on growth.

An experiment similar to the one described above was performed by McCollum and Davis (146). They compared the biological properties of corn-meal, wheat, wheat-embryo, rye, and rolled oats with dry pig-heart or kidney. The experiments, which were carried out on rats, demonstrated the beneficial effect, on growth, of corn-meal and wheat-embryo, the addition of rye had a slight favorable effect, but whole wheat-grain and rolled oats had practically no effect. The addition of pig-heart had less effect than the addition of kidney. These experiments show that food-stuffs known to contain a relatively large amount of vitamine stimulate growth, in rats, better than do those that contain relatively little vitamine.

As the results so far obtained sufficiently demonstrate the importance of vitamines for the growth of rats, Macallum and I have started new experiments with diets including different fractions from yeast. These experiments are now in progress.

XI. GROWTH IN PLANTS

Bottomley (147, 148) is the only one who claims to have demonstrated the importance of vitamins for the growth of plants. He was able to isolate, from bacterized peat, a substance which proved to be a powerful stimulant of the growth of plants. This substance was obtained from a fraction which corresponds entirely to the vitamin-fraction.

XII. INFLUENCE OF DIET ON THE GROWTH OF TUMORS

The results obtained thus far by applying the Osborne-Mendel diet, or any other similar vitamin-free diet, to animals bearing tumors have not justified the hope we had for this method. One is able partially to arrest the growth of a tumor on such a diet, but the avidity of the tumor for food is so great that its growth proceeds on a diet which is entirely inadequate for the growth of the animal. Some of the recent papers illustrate this point very well. I was able to show (144) that in chickens, on normal diets and inoculated with Rous's spindle-cell sarcoma, there were higher percentages of "takes" and larger tumors than in chickens fed on unpolished rice even after addition of yeast or sarcoma extract. Different results were obtained with diets of polished rice. Here the tumor did not "take" at all; whereas, in birds fed on polished rice plus yeast, tumors developed in a large percentage of cases. On these restricted diets no metastases were ever noticed. Food containing vitamins undoubtedly had marked influence on the growth of the tumors. Rous (149) has also studied the influence of simple underfeeding and of Osborne-Mendel diets on rats. Flexner-Jobling carcinoma was not affected by underfeeding after the tumor had been growing for some time. Spontaneous tumors of mice were affected by restricted diets if the feeding of such diets was started before the inoculation.

Two explanations for these results are available: When the organism is weakened the tumor does not grow as well as it does when the organism is more vigorous; in the case of a vitamin-free diet, the tumor does not receive essential specific nutrients. A great difficulty in such experiments is the fact that it is practically impossible to find a vitamin-free diet or a vitamin-poor diet on which

the animals can live in good condition. A second serious difficulty is the avidity of the tumor for food and the difficulty of rendering the animal itself vitamine-free before the tumor is actually inoculated. We saw that the tissues of pigeons that died from beriberi contained a certain amount of vitamine. However, it seems worth while to try the diet of unpolished rice, which is a complete diet, in human cancer, especially in rapidly growing inoperable cases.

Centanni (150) conducted experiments similar to those just referred to. He used what he called an aviride diet (seeds, dry fruit), in which the products of cellular activity are at a latent stage. This diet had a marked inhibitory effect on adenoma in mice, a slight degree of inhibition persisting even when fresh food was given. Van Alstyne and Beebe (151) have reported results of experiments with non-carbohydrate diets on the growth of sarcomas in rats. They found that the sarcoma grew very much less on a diet of casein and lard than on a diet of casein, lard and lactose.

The foregoing results led Benedict and Lewis (152) to investigate tumor-growth in rats with phlorhizin glycosuria. In such cases the growth of the tumor was markedly inhibited. A similar but not so striking a result was also obtained with a few human cases. At present a satisfactory explanation for these results is not available.

Further proof of the importance of specific food supplies for the growth of tumors is afforded by the results of experiments relating to transplantation of tumors into foreign species. That this is impossible, as a rule may be learned from recent papers by Rondoni (153) and Nasseti (154). The transplantation of tumors into foreign species has been conducted successfully, thus far, by one of the following three methods (*A-C*).

(*A*) Murphy (155) was able to implant rat tumor into a chicken embryo, which demonstrates either that the organism at a growing stage possesses the necessary specific substance for the growth of a tumor, even from a foreign species, or that resistance to tumor growth is less at embryonic stages than at later ones. That both factors play important rôles we shall see presently. (*B*) Murphy and Morton were able (156) to induce tumors to grow in foreign species by reducing the activity of the leucocytes with X-rays. (*C*)

I have shown (157) that mouse tumor can be successfully implanted in rats, for three generations, if the rats are fed on mouse-tumor tissue. The latter result demonstrates the necessity of a specific food supply for tumor growth. The fact is demonstrated, also, by the inhibition of tumor-growth in pregnant animals, as has lately been shown by v. Graff (158).

That there are substances which stimulate growth we know from the activity of glands of internal secretion, especially of the anterior lobe of the pituitary. Robertson and Burnett (159) have found that emulsions of anterior lobe of this gland stimulate very markedly the growth of primary carcinoma in rats, whereas liver emulsion had not the slightest effect. I should like to point out, here, the necessity of further research regarding the activity of pituitary gland (anterior lobe) on cancer. We know that this gland is important in regulating the growth of the organism. When the anterior portion of the gland is extirpated from animals, they remain stunted. This is also true, of course, of other glands of internal secretion but it is not so marked as in the case of the pituitary. On the other hand, the occurrence of adenomas of the anterior lobe is not infrequent. As a consequence of this abnormality, there is increased activity of the anterior portion of the pituitary. In children such abnormality produces symmetric exaggerated growth of the individual (gigantism). Between the age of 20-30 such general growth is no longer possible and the adenoma produces exaggerated growth of the bones of the extremities and the skull (acromegaly). It is conceivable that, in later life, when gigantism and acromegaly are no longer possible, increased activity of the pituitary gland may be responsible for the production of tumors.

When one considers that the symmetrical orientation of organs in a growing embryo must be accomplished by chemical substances and that growth in a seed is brought about by a renewal of enzymic activity, which transforms inactive substances (possibly vitamines) into new substances which stimulate cell division, one is not far from a chemical conception of cancer etiology.

The idea that the etiology of tumors can possibly be explained by the existence of specific growth-promoting substance received a new impetus from Rous's discovery of tumors, in fowls, that could

be propagated by cell-free filtrates. There seems to be a tendency to regard such tumors as entirely different from the known tumors in mice, rats and men. This may be true for the tumors in mice and rats, from which there are rarely metastases and which, as a rule, are entirely encapsulated. But we do not know whether very malignant human tumors, which are metastatic, can be propagated by means of a cell-free filtrate. On the contrary, the tumors in fowls seem to resemble human rather than any other experimental tumors.

As to the etiology of avian tumors two possibilities exist at the present time. They are either caused by a "filter-passer," as pointed out by Rous, or they are caused by a very unstable chemical substance, of a nature similar to that of vitamines. At present there is only a faint hope for a demonstration of the existence of such an unstable substance, but the problem was attacked by me, in collaboration with my late assistant, Mr. Drummond, in an investigation of the chemical composition of tumor extracts. I am also aware of the fact that no comparison can be made between the composition of the tumor, which consists of connective tissue, and of other tissue. Normal breast muscle of the fowl was taken as a control. At present I am comparing the chemical composition of spindle-cell sarcoma and osteochondroma, with the hope that new chemical substances can be isolated which will prove to be fragments of the hypothetic active substance.

I have recently found (160) that the blood of tumor-fowls (spindle-cell sarcoma) shows a very marked diminution in total nitrogen; as a rule, about 20-30 percent. This difference is not due to a change in the concentration of the serum, for one gets much less blood from tumor animals. It is surprising that this chemical change occurs a few days after the inoculation of the tumor, at a time when there is not the slightest trace of a tumor. At present I am investigating which fraction of the serum-protein is affected. Whether these chemical changes will throw any light on the etiology of the avian tumors we do not know, but they already constitute a perfect method for the diagnosis of these tumors. In human cases, however, such changes in the protein content of the serum could not be detected. This negative result is very likely due to the fact that the chemical changes in the serum

can be at best only very slight, since human tumors are very chronic. In fowls the whole blood was drawn and analyzed; and, as the duration of the disease is only a few weeks, very much more marked changes would be expected for fowl tumors than for tumors of human origin.

XIII. CONCLUDING GENERAL CONSIDERATIONS

We see, from the present review, that the establishment of the existence of a new group of important substances, the vitamins, has stimulated a great deal of work in this connection. As the numerous papers are scattered among many different journals, only a certain amount of the available data could be reviewed.

I have been severely criticized along two lines. In the first place objection has been taken to the fact that a name was given to these substances before they were isolated in a pure chemical condition. To this objection I answer that, besides the fact that we already know something about the vitamins from the chemical point of view, it has always been customary in physiological chemistry to give names to substances which exercise definite chemical influences, whether their chemical constitution is understood or not. Ferments, hormones and products of internal secretion, are among "substances" the chemical nature of which has not as yet been ascertained. As a matter of fact three-fourths of biological chemistry deals with this kind of "substances." As I use the term "vitamins," it indicates merely a group of chemical substances which possess every analogy to the already well-known class of nitrogenous bases, the members of which are precipitated by phosphotungstic acid or similar reagents, and which are thrown down, in the mercuric chloride fraction, and by silver nitrate and baryta. So far no other reagents have proved of any value for their isolation. Detailed knowledge of vitamins is now only a question of time and improved methods.

A second important objection to my views in this connection is the fact that many diseases have been included amongst the "avitaminoses," for which there may be other etiologies than deficiency of vitamins in the diet. There may be other etiologies for some of these diseases, it is true, but as the followers of other ideas have

entirely failed to throw any light on the etiology of pellagra, sprue, rickets, or spasmophilia, it seems advisable to direct them into another far more promising direction. My hypothesis on pellagra has already met with practical success; and rickets will very likely follow, when some of the clinicians test the new hypothesis. The only position that may have been unwarranted, I admit, was that of ascribing to cancer a possible chemical origin. My justification here is the fact that we are not making any headway in our knowledge of cancer by working with any of the existing hypotheses. My ideas have been found, by several authors, to be of value in general nutrition. It would be impossible to quote here all the available references but I should like to mention especially the papers by Melocchi (161), concerning general nutrition; by Friedenthal (162), regarding the feeding of infants; by Sternberg (163), on the vitamins in connection with appetite; and by Kunert (164), on the influence of deficient food on teeth.

Every observer who has studied experimental avitaminoses has noticed the diminished resistance of the animals to bacterial infections. Hüsey (165) has reported very good results, with a preparation from rice-polishings, in the treatment of weakness and inanition in women. Reach (166) investigated the resistance of mice, on different diets, against picrotoxin, a poison which produces spasms. Regarding the action of this drug on the central nervous system, the animals were found to be more resistant on a meat diet than on a bread diet. Reach accepts the existence of unknown favorable substances in the food.

Every investigator who has studied experimental deficiency-diseases has noticed the diminished resistance of the treated animals to bacterial infections. Some data are already available for men. Peiser (167) pointed out the importance of different fats and oils in infant nutrition and their protective power against infections. Morrison (168) sees great danger in restricting the diet of typhoid patients. Thomas (169) found, clinically, a diminished degree of immunity in children on chronic deficient nourishment. Rénon (170) suggested that there is diminished immunity to tuberculosis on diets containing insufficient quantities of vitamins. He proposed to investigate the influence of whole-meal bread and of

white-bread, and other vitamine-containing and vitamine-poor diets, on cases of human tuberculosis of the lungs and on experimental tuberculosis in animals.

The chief investigation on this phase of the general subject was carried out, in the mines in the Rand, by Macaulay (48) who found that the occurrence there of an epidemic of scurvy (which was due, in his opinion, to the use of over-milled maize as staple food) had, as sequelæ, large epidemics of pneumonia and meningitis, and increases in the number of cases of tuberculosis. Especially in pneumonia the administration of pneumococcic vaccine was applied without success. Macaulay then suggested that the maize should be less extensively milled. The execution of this suggestion proved to be a great success, for not only did scurvy disappear but also with it the occurrence of pneumonia. These data possess a great deal of importance since the results were obtained for a large number of cases: 2251 cases of pneumonia were reported in the year 1908, with 686 deaths, which gave an incidence of 72.93 per 1000 and a death rate of 22.23 per thousand workmen. The incidence of scurvy in one mine among the natives, for instance, was over 100 cases out of 700 natives.

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BIOCHEMISTRY OF COD-LIVER OIL.

(Preliminary note)

CASIMIR FUNK

INTRODUCTION. It is very surprising that the chemistry and physiology of cod-liver oil have been neglected to such a degree that the author was able to find only a few papers dealing with the subject.

In 1888, Gautier and Mourgues (1) showed that even the refined yellow oil contains a small quantity of organic bases (which were then considered to be ptomaines or alkaloids). Their technic was as follows: 100 k. of yellow cod-liver oil were extracted with the same volume of 33 percent alcohol, to which 4 gm. of oxalic acid per l. were added. The aqueous-alcoholic extracts were saturated with calcium hydroxid, filtered and evaporated *in vacuo*, at 45° C. Toward the end of the distillation, precipitated calcium carbonate and calcium hydroxid were added, the mixture evaporated to dryness, and the residue extracted with 90 percent alcohol. From this alcoholic extract the alcohol was removed *in vacuo*, water and strong caustic potash were added to the residue, and the alkaline mixture was extracted with ether. To the ethereal extract, a sol. of oxalic acid in ether was added; this precipitated the bases as oxalates. The yield was 52-65 gm. of oxalates from 100 k. of cod-liver oil. The oxalates were dissolved in dil. caustic potash sol., and the free bases separated, as an oil, on the surface of the liquid. The oil was removed and dried over freshly calcinated potash. In this way 0.35-0.5 gm. of dry substance was obtained.

Subjected to fractional distillation, products were obtained as follows:

- (a) Between 87-90°: Butylamin
- (b) Between 96-98°: Amylamin
- (c) Under 100°: Hexylamin
- (d) Between 198-200°: Hydrotoluidin

The distillation was continued to 215° . After cooling, the dark brownish residue was extracted with ether. The ethereal extract was evaporated and the residue dissolved in dil. hydrochloric acid sol. To this sol., platinic chlorid was added. The resulting precipitate was the chloro-platinate of *aselin*, an alkaloid of the composition indicated by the formula $C_{25}H_{32}N_4$, present in the original oil in small quantity. From the mother-liquor the chloro-platinate of *morrhuin* was obtained, to which the formula $C_{19}H_{27}N_3$ was ascribed. It is possible that these two substances were secondary products of the distillation.

A second paper dealing with this subject was published by Iscovesco (2), who claims to have isolated a lipid from cod liver that possesses all the known therapeutic properties of cod-liver oil.

The writer has been working on this subject during the past year in the hope of isolating vitamine-like substances that might account for the action of the oil in curing rickets and accelerating growth. The results of the preliminary work are given below.

Regarding the action of cod-liver oil there are two distinct views: that of the writer (3), who attributes the action to the presence in the oil of a vitamine-like substance; and of Osborne and Mendel (4) who regard the action as due to the special nature of the fats in the oil. The work of Gautier and Mourgues, and that of the writer, show that cod-liver oil contains a certain amount of organic bases, a fact which must be taken into account.

If the writer's view is correct, it might be advisable to administer, in rickets, cod-liver oil that is less purified than that used at present. Also, it should be possible to administer an extract of the organic bases without the oil. Such products have been obtained and will be tested on animals at the earliest opportunity. As experimental animals, chickens will be used, which develop in captivity, on a uniform diet, a condition resembling rickets. Some of the results obtained with cod-liver oil have already been published elsewhere (3).

The cod-liver oil subjected to fractionation was a very dark crude oil. A second sample of crude oil, though lighter in color, gave much less extractive material than the darker one. In the first case the oil was extracted by a method very similar to that

used by Gautier and Mourgues; and the extract was worked up by ordinary methods for the separation of organic bases. In the second case the oil was extracted with dilute sulfuric acid sol., and the phosphotungstate precipitate obtained was worked up by the acetone method—which has been used successfully in our work on yeast (5). Substances were separated in each fraction, but the quantity of each product was so small that the work will have to be repeated with more material.

EXPERIMENTAL. I. The cod-liver oil was entirely soluble in ether, acetone, ligroin and chloroform, and partially so in benzene. With alcohol alone, or with alcoholic mercuric chlorid sol., a slight precipitate was noticed. About 23.5 k. of the oil were used. Each k. was extracted with a sol. of 660 cc. of abs. alcohol and 50 cc. of conc. hydrochloric acid made up to 2 l. with water. Each k. was extracted three times, a third part of the sol. having been used in each extraction. The extracts were isolated in a separatory funnel, and evaporated *in vacuo*. The residue, which had an agreeable smell, was dissolved in alcohol, filtered from a precipitate which consisted mainly of inorganic salts, and the sol. evaporated again. Oil separated, which was twice washed with water and then hydrolyzed with 5 percent sulfuric acid sol. The watery extract was precipitated with 5 percent sulfuric acid sol. containing 50 percent of phosphotungstic acid. The precipitate was filtered, washed with dilute sulfuric acid sol., and dried. It weighed 969 gm. when nearly dry.

Treatment of the phosphotungstic acid precipitate. The precipitate was decomposed with 2 k. of baryta in a mortar. The phosphotungstate of barium was suspended in water and shaken. The combined filtrates were freed from baryta with sulfuric acid and evaporated *in vacuo*. The residue did not give a precipitate with alcoholic mercuric chlorid sol. No precipitate was obtained with silver nitrate and baryta. The sol. was freed from silver and baryta, and was reprecipitated with phosphotungstic acid. The resulting precipitate amounted to only 304 gm., consequently considerable decomposition of the nitrogenous substances must have taken place. This view is strengthened by the fact that the precipitate, decomposed with neutral lead acetate, gave a very heavy pre-

precipitate with mercuric chlorid. Before using this reagent, others were tried, but without success. The decomposed phosphotungstate precipitate had a very pronounced smell of nitrogenous bases. The final sol. of free bases was precipitated with mercuric chlorid in alcoholic sol. Both the precipitate and the filtrate gave crystalline chlorids after elimination of mercury and evaporation.

Treatment of the phosphotungstic acid filtrate. The filtrate was freed from phosphotungstic and sulfuric acids by means of neutral lead acetate. The filtrate, freed from lead with hydrogen sulfid, was conc. *in vacuo*. It was thought likely that it contained a large amount of amino-acids derived from liver tissue. The whole sol. contained 4.5 gm. of nitrogen and only 0.24 gm. of amino-nitrogen, as determined by Van Slyke's method. The residue, after evaporation, was entirely soluble in alcohol with the exception of a small quantity of inorganic material. The liquid was freed from chlorid with silver acetate, and the silver removed with hydrogen sulfid. The residue, when dissolved in alcohol and slowly evaporated, gave a crystalline substance. This will be investigated in the near future.

Treatment of the fatty residue obtained from the evaporated alcoholic-aqueous extracts. This oil was hydrolyzed with 5 percent sulfuric acid sol. for 2 hr. The filtered liquid, which smelt like herring, was precipitated with phosphotungstic acid; 19.5 gm. of dry precipitate were obtained.

Treatment of oil extracted with dilute alcohol. The extracted oil was hydrolyzed for 2 hr. with 5 percent sulfuric acid sol. The acid extracts were precipitated with phosphotungstic acid; 186 gm. of precipitate were obtained.

2. About 25 k. of the same supply of dark oil were extracted in portions of 2 k. each with 2 l. of 10 percent sulfuric acid sol. for 2 hr. on a shaking machine, and then left over night. The extracts were isolated in a separatory funnel and precipitated with phosphotungstic acid. The resulting precipitate (dry), which weighed 877 gm., was extracted with acetone and 57.2 gm. of insoluble fraction obtained. A second extraction of the oil, as above, yielded 240 gm. of phosphotungstate but only 4 gm. of the acetone-insoluble fraction.

Treatment of the acetone-insoluble fraction. The 61.2 gm. of material insoluble in acetone, obtained by the above mentioned treat-

ment, were treated in a mortar with 150 gm. of neutral lead acetate and shaken on a machine for 1 hr. Alcohol was added to render the precipitate more insoluble; the liquid was filtered. The filtrate was freed from excess of lead and evaporated. The resulting white residue was dissolved in water, and alcohol added. Gelatinous material separated out, which was filtered off; 1.3 gm. was obtained. The aqueous filtrate from this substance was evaporated *in vacuo*, the residue dissolved in alcohol and precipitated with alcoholic mercuric chlorid sol. The resulting precipitate was decomposed with hydrogen sulfid and the filtrate evaporated to dryness. The residue was redissolved in water, and the liquid freed from chlorid by means of silver acetate. The filtrate, freed from silver, gave 3 gm. of substance, which is now being carefully investigated.

The mercuric chlorid filtrate was freed from mercury with hydrogen sulfid, and evaporated; the residue was dissolved in water and freed from chlorid with silver acetate. The filtrate from the silver sulfid gave, after evaporation, 0.1 gm. of substance.

Treatment of the acetone-soluble fraction. The acetone sol., obtained by the above mentioned treatment, was diluted with water, decomposed with 2 k. of neutral lead acetate, shaken for 1 hr., and filtered. The precipitate was again suspended in 30 percent acetone and filtered. The combined filtrates were freed from excess of lead and evaporated *in vacuo*. The residue, which did not crystallize, was dissolved in water and precipitated with alcoholic mercuric chlorid sol.: 99 gm. of precipitate (dry) were obtained. Both the precipitate and the filtrate were freed from mercury and evaporated; both yielded small amounts of crystalline hydrochlorids. The filtrates from the hydrochlorids were fractioned in the usual manner with silver nitrate, and with silver nitrate and baryta. These fractions, which gave only exceedingly small amounts of different substances, will be investigated later when larger quantities of material are available.

SUMMARY. With the idea that the therapeutic action of cod-liver oil is not due to peculiar fatty constituents in the oil, but to the presence of nitrogenous substances, a separation of the latter from the oil was effected. The raw material used was crude cod-liver oil, since this is richer in organic bases than the purified variety.

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THE PROBLEM OF REJUVENESCENCE IN PROTOZOA*

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It is a pleasure to accept the invitation to present a summary of the results derived from our genetic cultures of *Paramæcium* at Yale, with special reference to the bearing of this and other work on the problem of rejuvenescence in protozoa; for, it seems to me, the problem has now passed successfully through the periods of youth and adolescence, and is approaching that of maturity, when we may confidently expect the production of some conclusions of general significance.

Although the problems of protoplasmic senescence and the function of conjugation have afforded the stimulus for investigations on the life history of infusoria since Ehrenberg, nearly a century ago, theorized on the potential 'immortality' of those forms, we may take, as the point of departure for our present brief review of the subject, the classical experimental studies of Maupas. As is well known Maupas' studies afforded a wealth of data, all of which indicated that continued reproduction by division results in degeneration and death, and seemed to place the conclusion, that conjugation is a *sine qua non* for the life of infusoria, upon a firm empirical basis.

A series of important investigations by Hertwig and Calkins confirmed Maupas' general conclusion that infusoria, after a more or less definite number of divisions, degenerate and finally die if conjugation is prevented. Calkins, however, made the significant discovery that artificial stimuli of different kinds may, for a time, be substituted with success for conjugation since, by the opportune use of artificial stimulation, he was able to prolong the life of one culture of *Paramæcium caudatum* to the 742d generation.

* Presented at the symposium on protozoology. Amer. Ass'n. Adv. Science, Berkeley, Cal., August 5, 1915. For a review of the earlier work on this subject, at Yale, see BIOCHEMICAL BULLETIN, 1912, i, p. 396.

Enriques studied the same general problem and reached the conclusion that the degeneration and death of infusorian cultures was due to bacterial poisons, because he succeeded in breeding *Glaucoma scintillans* for 683 generations without signs of degeneration when he took measures to eliminate this factor. Whether this was the crucial factor in his method is open to question, but the significant fact remains that his animals survived nearly twice as long as those of earlier workers, without conjugation or artificial stimulation, thus suggesting that, if suitable conditions are supplied, reproduction by division can proceed indefinitely.

At this point I took up the problem and first investigated the possibility that the degeneration observed in the previous investigations was induced by too great uniformity in the conditions of culture, or by the culture-medium being deficient in something essential for the continued well-being of the organisms. A race of *Paramæcium aurelia* was isolated in 1907 and bred on infusions of various materials found in the natural environment of the animal, while a sub-culture was subjected to the relatively constant hay-infusion culture-conditions generally employed. The result was that the cells bred in the constant hay-infusion medium died out after a typical Calkins cycle, while those bred on the 'varied-environment' medium did not pass through periods of marked physiological depression or show morphological changes which could be interpreted as abnormal. This race is still, after more than eight years in culture, in a normal condition, having attained over 5250 generations without conjugation or the use of artificial stimuli.

The success with the varied culture medium naturally led to the question whether the longevity of *Paramæcium*, on a varied environment, is dependent upon intrinsic stimuli from the frequent changes of the medium, or whether a constant medium of hay-infusion is unfavorable because it lacks some elements which are essential for the continued existence of the organism. Accordingly,¹ a sub-culture of this race was bred for a period of nine months on a constant culture-medium of beef-extract. The continued health of the organisms on this constant medium throughout the experiment, which was continued sufficiently long to include a Calkins cycle, if such

¹ Woodruff and Baitzell: *Journ. of Exp. Zool.*, 1911.

was inherent, indicated that it is the composition of the medium, rather than the changes in the medium, which is conducive to the unlimited development of this race without the necessity of conjugation or artificial stimulation.

From a study of various species of hypotrichous infusoria, as well as the main culture of *Paramæcium aurelia*, it was found that minor periodic rises and falls of the division-rate occur, from which recovery is autonomous. These fluctuations were termed 'rhythms' and contrasted with the so-called cycle, which comprises a varying number of rhythms and, according to Maupas and Calkins, ends in the death of the race, if conjugation or artificial stimulation is not resorted to.

The problem of rhythms was then studied intensively.² It was found that the subjection of the culture to the most constant environmental conditions failed to eliminate the rhythms and thus to resolve the graph of the multiplication rate into an approximately straight line; but, instead, the rhythms appeared slightly more pronounced. It was also found, from a study of the temperature coefficient³ of the rate of reproduction of the culture, that this is influenced by temperature at a velocity similar to that for a chemical reaction, except when the rhythms interfere. Thus, it is apparent that there are inherent rhythmical changes in the phenomena of the cell which produce slight fluctuations in the division-rate.

The results, then, from the study of this pedigreed race of *Paramæcium aurelia* led us to conclude that this organism, when subjected to suitable culture conditions, has the power of unlimited reproduction by division without conjugation or artificial stimulation; the only necessary variation in the rate of reproduction being the normal minor periodic rise and fall of the division-rate, due to some unknown factor in cell phenomena, from which recovery is autonomous (rhythm).

Calkins,⁴ however, did not share this optimism and sought the explanation, of the diametrically opposite results derived from his and from our cultures of *Paramæcium*, in variations in the tendency

² Woodruff and Baitsell: *Journ. of Exp. Zool.*, 1911.

³ Woodruff and Baitsell: *Am. Journ. Physiology*, 1911.

⁴ Calkins: *Journ. of Exp. Zool.*, 1914.

to conjugate, which he and Jennings had found to exist in different races of this organism. Thus, Calkins emphasized the fact that he could readily induce conjugation in his culture, whereas experiments to secure conjugation in our cultures were without effect. He, therefore, stated that "the two races cannot be compared in regard to vitality, since normal conjugation was prevented in the conjugating race, whereas in the non-conjugating race there has been no artificial prevention of a normal process."

With this issue raised, it was essential to determine whether our race was actually non-conjugating. Accordingly, a more extensive series of mass cultures were started from it, with the result that conjugants were finally secured, thus demonstrating that this race is a conjugating race when the proper conditions for conjugation are realized. Therefore, there is no evidence extant that a non-conjugating race of *Paramœcium* exists.

In a recent paper, Calkins⁵ states that possibly his terms "conjugating" and "non-conjugating" were not happily chosen, and that he merely meant to indicate that some races are more prone to conjugate than others. Admitting this interpretation of his terms, they express a fact. But this interpretation begs the question which his suggestion was advanced to explain.

With this theory eliminated, the results derived from this culture demonstrate, we believe, that the very limited periods in which Maupas, Calkins, and others observed degeneration, have no significance for the question as to whether degeneration and death are inevitable results of reproduction without conjugation. In other words, this one positive result from this race outweighs all the negative evidence derived from work on the infusoria, and justifies the statement that these organisms can live indefinitely, when subjected to favorable environmental conditions, without conjugation or artificial stimulation.

With conjugation eliminated as a necessary factor in the life history, obviously the next point to be elucidated, if possible, was the underlying factor inherent in the cell, the physiological expression of which is the rhythm. Although morphological or physiological variations that could be interpreted as the result of degeneration

⁵ Calkins: *Am. Naturalist*, 1915.

were never observed in this race of *Paramæcium*, we early noted "that various nuclear changes which are not at present recognized occur normally in the life history of *Paramæcium*"; and we suggested that possibly, when conjugation is prevented, a reorganization of the nuclear apparatus within the individual cell occurs.⁶ Erdmann independently reached an essentially similar position from a consideration of the published data on this culture and a critical study of infusorian life histories; and further, in an experimental study of *Amæba diploidea*, suggested that a relation exists between sexual phenomena and rhythms.⁷ Accordingly, we collaborated in a study of the *daily* cytological changes of this race of *Paramæcium* during a period of six months, and discovered that the rhythms in the division-rate are the physiological expression of internal phenomena which involve the formation of a complete new nuclear apparatus, by a definite sequence of normal morphological changes that simulate conjugation.⁸ This nuclear reorganization, which we term *endomixis*, consists, in essence, of a gradual disintegration and absorption of the macronucleus in the cytoplasm. Simultaneously, a multiplication of the micronuclei is in progress. Certain of the resulting micronuclei degenerate while the remaining one (or two) form the new macronuclear and micronuclear apparatus. This results in the reorganization of the cell without the fusion of two animals.

An essential morphological difference between endomixis and conjugation is the absence of the third micronuclear division, which, in conjugation, forms the stationary and migratory micronuclei; and, of necessity, the non-formation of a *syncaryon*. After conjugation the reorganized cell has a new macronuclear and micronuclear apparatus, composed of combined material from the conjugants, while, after endomixis, the reorganized cell has a new macronuclear and micronuclear apparatus composed of material from its own micronuclei. In a word, the essential distinctive features of endomixis are the absence of the third micronuclear division and

⁶ Woodruff: *Amer. Naturalist*, 1908.

⁷ Erdmann: *Ergeb. d. Anat. u. Ent.*, 1908; *Archiv. f. Protistenk.*, 1913.

⁸ Woodruff and Erdmann: *Proc. Soc. Exp. Biol. and Med.*, 1914; *Journ. Exper. Zool.*, 1914; also Erdmann and Woodruff: *Biol. Cent.*, 1914.

the absence of the introduction of foreign nuclear (and cytoplasmic) material into the cell.

For reasons advanced elsewhere, we hold that endomixis is not parthenogenesis, but it is not necessary at this time to enter into a more or less academic discussion in regard to the exact classification of endomixis among *Entwicklungs-erregung* phenomena.

In the light of the discovery of the details of endomixis, by the daily study of pedigreed cells of this race, a survey of the cells, which had been preserved at intervals during the previous seven years of its life, revealed a number of the crucial stages of endomixis, thus showing that the process has been in progress ever since the race has been bred, and is not, as Hertwig⁹ suggests, a development during long subjection to culture. That it is not even a peculiarity of this race is evident from the fact that we have found endomixis in four other distinct races selected at random—three from America and one from Germany. Further, Hertwig, in 1889, incidentally noted in a mass culture, in which conjugation had not been observed for a long time, certain animals whose nuclear structure apparently indicated isolated stages of the process which have been elucidated in our cultures. Therefore, it seems well established that endomixis is of general and probably universal occurrence in *Paramacium aurelia*. It also occurs, with essentially similar features, in all the races of *Paramacium caudatum* which we have studied.¹⁰

Now, in regard to the significance of endomixis from the standpoint of our subject—rejuvenescence in protozoa: It is clear that the cycle emphasized by Maupas, Calkins and others, is merely a phantom which has continually receded as each successive investigator has approached the problem with improved culture methods, until it has vanished with this eight-year-old culture. What remains then is the rhythm and in the light of endomixis—the underlying cytological phenomenon of which the rhythm is an outward physiological expression—the whole problem takes on a new aspect. The cell automatically reorganizes itself periodically by a process which, in its main features, simulates conjugation, but without a contribution of nuclear material from another cell.

⁹ Hertwig: *Biol. Cent.*, 1914.

¹⁰ Erdmann and Woodruff: *Journ. Exp. Zool.*, 1916.

At the present stage of our knowledge, the rhythm may perhaps be considered as an expression of a sort of temporary "senescence," for which is provided an internal, automatically working, antidote in the form of endomixis. But, if one considers this as a "senescence"-phenomenon and endomixis a "rejuvenation"-phenomenon, then it is equally permissible so to consider the momentary fluctuating periods of anabolic and catabolic ascendancy in the metabolism of the cell. But I would point out that it is a case of trying to force new wine into old bottles in order to save an idea, and that this line of reasoning, pushed a little farther, approaches perilously near a *reductio ad absurdum*. One certainly must grant that the prevalent idea of "senescence" in infusoria is far removed from this subtle, automatically eliminated type which rhythms may indicate.

The work at Yale in the past has been to determine whether *conjugation* is a necessary factor in the life of infusoria; and we believe that 5250 generations without conjugation is *strong* evidence in the negative. But, obviously, because conjugation is not necessary in the life of *Paramacium* under favorable environmental conditions, it does not follow that conjugation is not necessary under other conditions, or that it does not have a "rejuvenating" function. There is nothing mutually exclusive in the *fact* that conjugation is not necessary and the *idea* that conjugation has a dynamic function when it occurs. In fact we have *always* leaned toward and definitely stated the view that conjugation probably has a dynamic function, which is important when the organisms are subjected to unfavorable conditions. Calkins has secured some evidence which indicates that, after conjugation, all the processes of the cell including reproduction proceed with greater vigor; and thus he substantiates the view of Bütschli, Maupas, Hertwig, and others. Jennings, on the other hand, definitely states that there is no evidence from his work that conjugation in the infusoria increases the reproductive power of, or rejuvenates, the organism physiologically in any way and puts all the emphasis on the side of variation and heredity.

However, since the cytological phenomena of conjugation, with the exception of syncaryon formation, are so similar in their broad features with those of endomixis, and since accelerated vital activities

including reproduction do *follow* endomixis, it seems reasonable to believe that accelerated vital phenomena follow conjugation—that is, that both processes, broadly speaking, “rejuvenate” the organism physiologically. Both processes afford opportunity for a rearrangement of the molecular constitution of the cell, conjugation affording amphimixis and endomixis affording *endomixis*.

To recent contentions that our conclusions were wrong, in regard to conjugation not being a necessity for the continued reproduction of infusoria, we would reply that endomixis is *not* conjugation; and no one had any other phenomenon than conjugation, involving syncaryon formation, in mind until the discovery of endomixis, in which a syncaryon is not formed. To say that endomixis fills essentially the same rôle as conjugation in the infusorian life-history is to beg the entire question. In a word, the whole aspect of the problem of senescence and rejuvenescence in protozoa has changed with our knowledge of endomixis. The question is now not whether conjugation is necessary—for it is not—but whether endomixis is necessary. If endomixis is necessary, as it may well be, and *if* one feels justified in considering the physiological phenomena which are synchronous with the start of endomixis as evidence of “senescence” and those synchronous with the end of endomixis as indicating “rejuvenation”—then, this is a radically *new* phase of the old idea of protoplasmic senescence and rejuvenescence in the infusoria.

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THE CHEMICAL CONSTITUTION OF STARCH

A review

ARTHUR W. THOMAS

The size and configuration of the starch molecule have been the subject of much chemical research since about 1836, when Payen¹ announced the chemical composition to be $C_6H_{10}O_5$. This is the empirical formula generally accepted at the present time. The real molecular structure is known to be many times this empirical unit, however. The highly colloidal nature of starch, the ease with which it can be removed from its solutions by merely forcing them through porous earthenware, and the extremely small influence which it exerts on the freezing point of its solvent, are facts that indicate a high degree of molecular complexity, which probably approaches that of the proteins.

The $(C_6H_{10}O_5)_n$ structure indicates that starch is an anhydride condensation product of glucose or maltose. This view is borne out by the fact that hydrolysis by acid or diastase yields glucose or maltose, respectively, as the end products. The contributions to our knowledge of the molecular weight have all, or nearly all, depended upon the study of the hydrolysis of starch by infusions of malt, a type of study which has naturally been fostered by the great fermentation industries.

In 1860 Musculus² noted that it was difficult, in fact quite impossible, completely to hydrolyze starch to maltose by means of malt. After much experimenting he announced, in 1878 (Musculus and Grueber),³ his view that starch must be a polysaccharide of a molecular size indicated by the formula $(C_{12}H_{20}O_{10})_{5-6}$. In 1879 O'Sullivan⁴ announced that the starch molecule is as large as

¹ Payen: *Ann. Chim.*, 1836, [II] lxi, p. 355; 1837, [II] lxxv, p. 225.

² Musculus: *Ann. Chim.*, 1860, [III] lx, p. 203; 1865, [IV] vi, p. 177.

³ Musculus and Grueber: *Bull. soc. chim.*, 1878, xxx, p. 54.

⁴ O'Sullivan: *Jour. Chem. Soc.*, 1879, xxxv, p. 770.

the molecular size $(C_{12}H_{20}O_{10})_6$, thus corroborating the work of Musculus.

Herzfeld,⁵ while not contributing directly to the knowledge of the size of the starch molecule, started a new line of thought, in which he pointed out that the hydrolysis progressed through a series of dextrans of diminishing complexity before, or in the course of, the conversion of starch to sugar, *i. e.*, starch, to soluble starch, to erythro-dextrin, to achroo-dextrin, to maltodextrin, finally to maltose. To maltodextrin he assigned the formula $C_{18}H_{36}O_{16}$. The discovery of maltodextrin was quite a significant step, for all subsequent work has depended on the study of just such substances—substances which combine the properties of sugar and of dextrin.

Brown and Heron⁶ found that the hydrolysis of starch by malt stopped when four fifths of its weight of maltose was formed, the remaining one fifth consisting of a dextrin. They accepted the theory of Musculus and Grueber, and of Herzfeld, that starch was hydrolyzed in successive steps to dextrin and to sugar. From their experiments with malt diastase they concluded, most naturally, that the starch molecule must be at least five times the size of the residual dextrin, and proposed $(C_{12}H_{20}O_{10})_{10}$ as the formula for starch, the simplest dextrin molecule being thought to be $(C_{12}H_{20}O_{10})_2$.

In 1885, Brown and Morris⁷ reported that a dextrin was always present as one of the hydrolytic products of starch, which, while not identical with the maltodextrin of Herzfeld, bore a resemblance to it. They assigned to this compound the formula $(C_{12}H_{20}O_{10})_2 \cdot C_{12}H_{22}O_{11}$. Inasmuch as this dextrin was difficult to hydrolyze they gave starch the formula $5(C_{12}H_{20}O_{10})_3$, in order to make it agree with the principles evolved from their earlier work. The $(C_{12}H_{20}O_{10})_3$ group was called amylin, the starch molecule consisting of four such groups arranged symmetrically about a fifth. Upon hydrolysis an amylin group was thought to split off as maltodextrin, leaving the other four as a more complex dextrin, the maltodextrin in turn splitting directly into maltose.

Brown and Morris⁸ determined the molecular weight of amylin

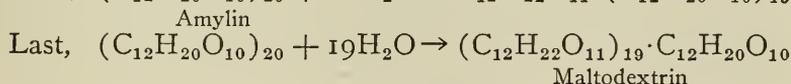
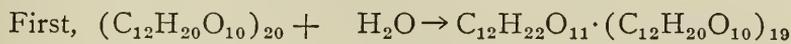
⁵ Herzfeld: *Berichte*, 1879, xii, p. 2120.

⁶ Brown and Heron: *Ann. Chem.*, 1879, cxcix, p. 165.

⁷ Brown and Morris: *Ann. Chem.*, 1885, ccxxxi, p. 72.

⁸ Brown and Morris: *Jour. Chem. Soc.*, 1889, lv, p. 96; *Berichte*, 1891, xxiv,

by means of its depression of the freezing point of water. Their experimental figure for the molecular weight was 6221, which agreed most closely with the formula $(C_{12}H_{20}O_{10})_{20}$, the molecular weight of which is 6480. They adhered to their theory that starch was composed of five amylin groups and represented the hydrolysis by these equations:



Brown and Morris concluded, from the results of their later work, that the maltodextrins split into smaller substances of varied composition. Two different maltodextrins were isolated by the authors, one with the formula $(C_{12}H_{20}O_{10})_2 \cdot C_{12}H_{22}O_{11}$ (maltodextrin) and another with the formula $(C_{12}H_{20}O_{10})_6 \cdot C_{12}H_{22}O_{11}$ (amylo-dextrin).

Scheibler and Mittlemeier,⁹ in 1890, discussed the hydrolytic products of starch and dextrin. One noteworthy feature of their paper was the preparation of the hydrazone of a commercial dextrin which, upon analysis, was found to have a composition indicated by the formula, $C_{96}H_{162}O_{80}N_2HC_6H_5$. This corresponds with $(C_6H_{10}O_5)_{16}$, which is somewhat similar to the amylo-dextrin reported by Brown and Morris.

Lintner and Duell¹⁰ claimed that, in its hydrolysis, the complex starch molecule split first into amylo-dextrin (better known at the present time as soluble starch), and that this soluble starch then broke down into three molecules of erythro-dextrin, which in turn split into three molecules of achroo-dextrin, the latter splitting into iso-maltose, iso-maltose changing to maltose. They determined the molecular weight of these substances by means of the freezing-point method of Raoult, with the following results:

Soluble starch	17,496	$(C_{12}H_{20}O_{10})_{54}$
Erythro-dextrin	5,850	$(C_{12}H_{20}O_{10})_{18} \cdot H_2O$
Achroo-dextrin	1,962	$(C_{12}H_{20}O_{10})_6 \cdot H_2O$

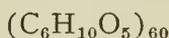
In 1897 a formula only one quarter as great as that of Lintner

⁹ Scheibler and Mittlemeier: *Berichte*, 1890, xxiii, p. 3060.

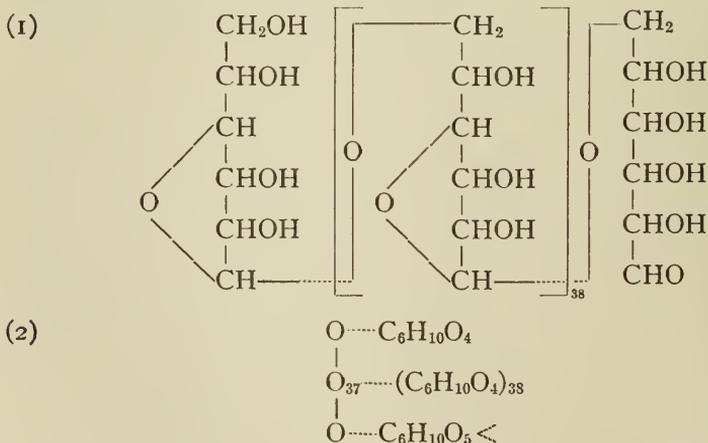
¹⁰ Lintner and Duell: *Berichte*, 1893, xxvi, p. 2533.

and Duell was proposed by Rodewald.¹¹ The determination of the molecular weight was accomplished by means of the lowering of the vapor pressure of water. The calculated value was 4370, equivalent to $(C_6H_{10}O_5)_{27}$; the correct weight for this formula is 4374.

This reduction in size of the proposed molecular weight of starch was reversed, however, two years later by Friedenthal,¹² who dissolved a commercial soluble starch (called ozone starch) in water and reprecipitated it with alcohol. This product showed, by the freezing-point method, a molecular weight of 9450, which, according to Friedenthal, corresponds with the formula



In 1899, Brown and Millar¹³ presented the results of several years of further work upon the hydrolytic products of starch. The main feature of their paper was the discovery of a reducing dextrin to which they gave the formula $39(C_6H_{10}O_5) + (C_6H_{12}O_6)$, or $40(C_6H_{10}O_5) + H_2O$. As the empirical formula indicates, it might be made up by the condensation of forty glucose molecules with the elimination of thirty-nine molecules of water, in accord with the appended structural formulas:



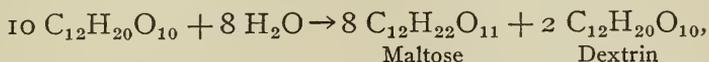
(< signifies a free aldehyde group)

¹¹ Rodewald: *Zeitsch. physik. Chem.*, 1897, xxiv, p. 193.

¹² Friedenthal: *Centralb. f. Physiol.*, 1899, xii, p. 849.

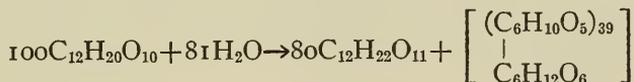
¹³ Brown and Millar: *Jour. Chem. Soc.*, 1899, lxxv, p. 333.

The ordinary combustion method would fail to reveal the presence of one H_2O -group in a molecule of this size. This difficulty was surmounted by the oxidation of this dextrin by mercuric oxide to a dextrinic acid, which, in turn, was precipitated by lime to form the calcium salt. This salt contained the theoretical amount of calcium, 0.3 percent; and, upon hydrolysis, yielded the correct amount of glucose. As previously mentioned, the earlier work of Brown and Heron resulted in the suggestion of the equation,



as a correct representation of hydrolytic changes.

Inasmuch as this newly discovered dextrin could not be represented by a formula simpler than $40 \text{C}_6\text{H}_{10}\text{O}_5 + \text{H}_2\text{O}$, it was necessary to modify the above equation to allow for it, as follows:



If this equation holds, then the starch molecule must be at least five times the size of the dextrin molecule. Now, the molecular weight of the dextrin is 6498 or $6480 + 18$; therefore, the weight of the starch molecule is at least 32,400, equivalent to $(\text{C}_6\text{H}_{10}\text{O}_5)_{200}$. (In 1889 Brown and Morris had found that the freezing-point method indicated a molecular weight, for soluble starch, of about 20,000 to 30,000.)

Since starch is non-reducing it has no free carbonyl group and, hence, the simplest manner, to quote Brown and Millar, "to express its constitutional form with due regard to all facts is to consider it made up of the residues of eighty maltan and forty dextran groups linked in a ring form by means of oxygen atoms. On hydrolysis the dextran complex is split off with the formation of stable dextrin, whilst the maltan part of the ring is attacked at the oxygen linkings of the C_{12} groups, hydrogen ions of reacting water moving in one direction and hydroxyl ions in another, thus forming by successive stages of hydrolysis, maltodextrins and maltose."

This reasoning seems to be logical and, if the experimental re-

sults have been correctly interpreted, the starch molecule may be correctly represented by their proposed configuration (Fig. 1):

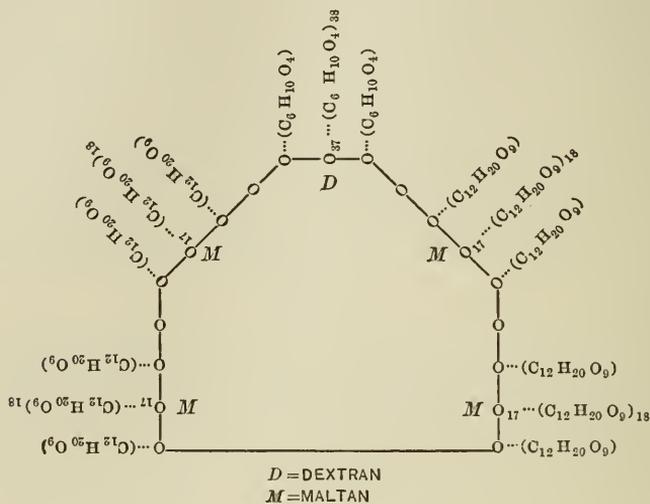


FIG. 1.

This structure has lately been attacked by Ling,¹⁴ who claims that the hydrolysis of starch by malt diastase does not produce eighty percent of maltose; maltodextrins being present. This statement, however, due to the great amount of evidence in favor of the production of eighty percent of maltose by the action of malt enzyme on starch, is open to question. Ling prefers to regard the starch formula as $(C_6H_{10}O_5)_n - (n-1 H_2O)$, similar to the $(C_6H_{10}O_5)_n + H_2O$ proposed by Kiliani,¹⁵ inasmuch as he considers carbohydrates to be derived from monoses by a series of condensations with the elimination of water. This view is at the present time quite general. As the proposed starch structure of Brown and Millar does not allow for this one molecule of water, their formula may, of course, possess other weak points.

In the year previous to the publication of the last mentioned work there appeared a paper by Johnson¹⁶ in which he proposed a molecular formula for starch. Experimental evidence is lacking,

¹⁴ Ling: Proc. 7th Internat. Congr. Appl. Chem., 1910, viB, p. 123.

¹⁵ Kiliani: *Chem. Zeit.*, 1908, xxxii, p. 366.

¹⁶ Johnson: *Jour. Chem. Soc.*, 1898, lxxiii, p. 490.

the structure being the result wholly of hypothesis. The author says that the schema was intended to convey, only in a *figurative* manner, the probable nature of the starch molecule. The formula is based upon the fact that starch must be a multiple of $(C_6H_{10}O_5)_4$, these groups being arranged as follows:

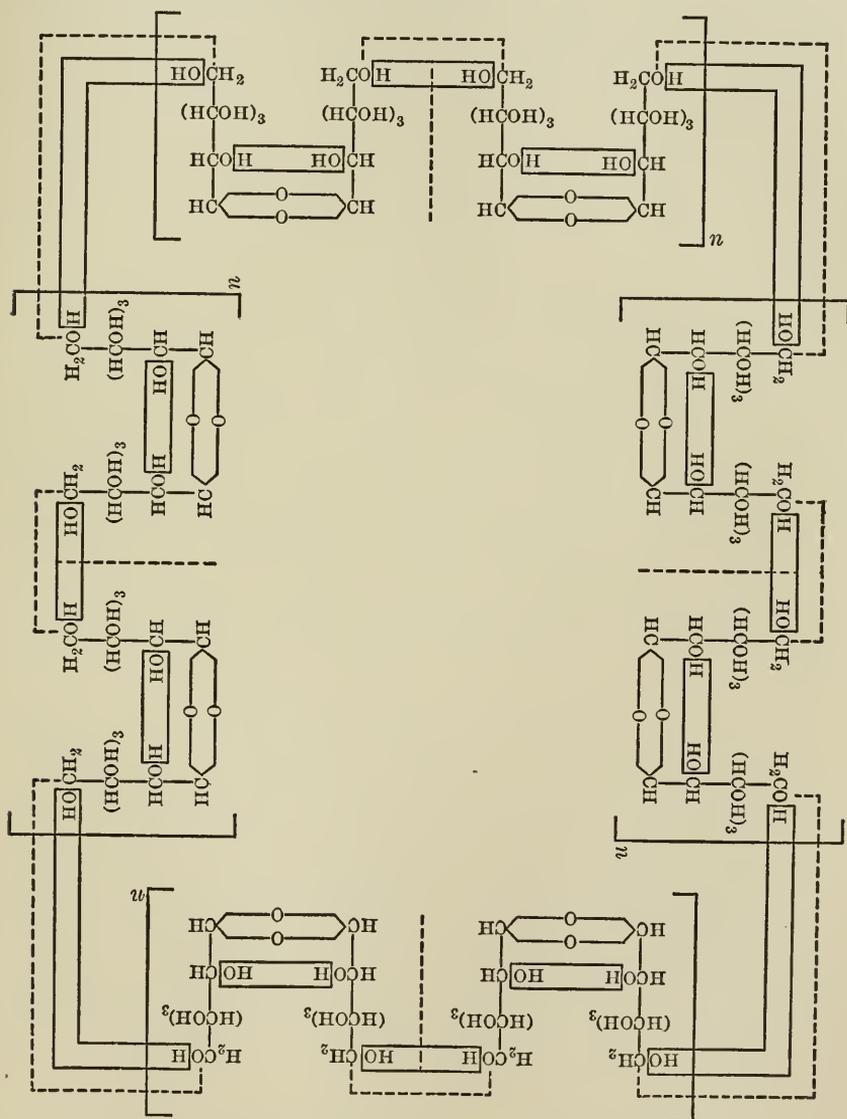


FIG. 2. Proposed structural formula for starch (Johnson).

To use Johnson's own words,—“As is seen, two dextrose molecules condense in a secondary group $(\text{HC.O} \overline{\text{H HO}}).\text{CH}$) whilst the maltose molecules condense in the primary group $(\text{H}_2\text{C.O} \overline{\text{H HO}}).\text{CH}_2$), the former becoming $\text{HC.O}—\text{CH}$ and the latter $\text{H}_2\text{C.O}—\text{CH}_2$. The two aldehyde groups of the original dextrose molecules condense in the amylin groups as is shown above. This explains the non-reducing character of the starch.”

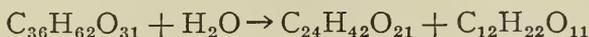
The suggested condensation of “primary” and “secondary” OH groups in each sugar molecule has no experimental justification. In my opinion it does not seem necessary to assume polymerization of the aldehyde groups to explain the non-reducing character of starch. The disaccharide sucrose is not a reducing sugar, yet we do not assume that the aldehyde groups of the constituent monoses polymerize with one another.

The latest attempt to construct a starch molecule, and probably the most painstaking of any previous hypothesis, was brought out by Synkiewski¹⁷ in 1902. His work consisted in studying the products of hydrolysis of starch by infusions of malt, with exhaustive investigation of the properties of the several dextrans isolated therefrom. The starting point in his work was the isolation of two dextrans, one called by him “protodextrin I” which was formed by hydrolyzing starch at ordinary temperature; and another, called “protodextrin II,” which was formed by the hydrolysis of starch at 78° C.

The preparation of protodextrin II was accomplished by allowing malt extract to act on starch at about 78° C. until all starch was hydrolyzed. The dextrin was then separated by evaporation of the water, extraction with dilute alcohol to remove soluble carbohydrate, and final precipitation with strong alcohol. The product was a white amorphous powder which, after ultimate analysis and by determination of the depression of the freezing point of water, showed a molecular weight and structure equivalent to $\text{C}_{36}\text{H}_{62}\text{O}_{31}$ or $(\text{C}_6\text{H}_{10}\text{O}_5)_6 + \text{H}_2\text{O}$. This is similar to the formula for the

¹⁷ Synkiewski: *Ann. Chem.*, 1902, cccxxiv, p. 212.

maltodextrin- α of Ling and Baker,¹⁸ the achroodextrin II of Lintner,¹⁹ and the maltodextrin of Brown and Morris.²⁰ This protodextrin was further hydrolyzed by malt extract; and, by a similar process, another dextrin was isolated from the hydrolytic products. This dextrin was termed γ -maltodextrin. By analysis and depression-of-the-freezing-point-measurements, its elementary constitutional formula was determined to be $C_{24}H_{42}O_{21}$. Synkiewski claims that this dextrin is identical with the maltodextrin- β of Ling and Baker, and the achroodextrin of Prior.²¹ Its formation from protodextrin II is represented by the equation



Hydrolysis of this maltodextrin yields the isomaltose of Lintner.

Protodextrin I was prepared and isolated in a manner similar to that for protodextrin II, with the exception that the malt extract acted in the cold. The molecular formula was found to be $C_{72}H_{124}O_{62}$ or just twice that of protodextrin II. Hydrolysis of this substance by malt yielded a sugar with the same formula as maltose, but, because of its higher optical rotation and lower reducing power than maltose, it was believed to be a polymer of the latter sugar and received the name "dextrinose."

The next part of this author's investigations consisted in the study of soluble starch (prepared by heating starch paste in an autoclave under pressure) or, as he called it, amylo-dextrin. He determined its formula to be $(C_{54}H_{90}O_{44})_n + \frac{3n}{2} H_2O$; and, by means of acetylation, found it to contain thirty hydroxyl groups. Years ago Schützenberger and Naudin²² analyzed an acetyl derivative of ordinary starch, with results, according to Synkiewski, that demonstrated the presence in ordinary starch of twenty-seven hydroxyl groups. Since the formation of maltose from this thirty-hydroxyl amylo-dextrin is complete, and since the hydrolysis of ordinary starch stops before all of it is converted to maltose, he concludes that the amylogen residues of the starch molecule (each of which contain

¹⁸ Ling and Baker: *Jour. Chem. Soc.*, 1897, lxxi, p. 517.

¹⁹ Lintner: *Zeitsch. f. d. ges. Brauw.*, 1894, p. 339.

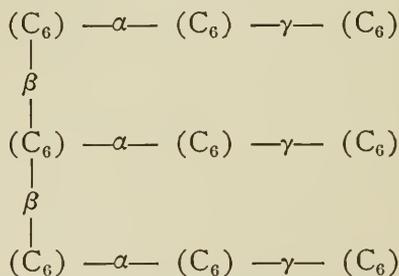
²⁰ Brown and Morris: *Jour. Chem. Soc.*, 1885, xlvi, p. 527.

²¹ Prior: *Bayr. Bierbr.*, 1896, p. 157.

²² Schützenberger and Naudin: *Ann. Chem.*, 1871, clx, p. 77.

three maltose residues), under the influence of malt extract, first split off these maltose residues as maltose molecules, provided they have been previously provided with hydroxyl groups. For each hydroxyl that the amylogen nucleus takes on, one maltose residue is ready to be split off by the diastase.

Upon the configuration of the amylogen residues rests the structure of the starch molecule. Synkiewski holds that the amylogen residue contains three different kinds of carbonyl linkings: the one which is readily hydrolyzed by malt, and yields maltose and protodextrin I, is called an α bond; the second, which is broken only by long action of malt diastase and finally yields glucose from the protodextrin, is called β ; and the third, which connects the glucose residues in maltose, is called γ . According to this scheme the amylogen nucleus may be represented by



Since starch consists of n amylogen residues connected by anhydride carbinol linkings, then, when an α -carbonyl hydrolysis takes place, $3n$ molecules of maltose are produced and n molecules of the protodextrin I. A β -carbonyl hydrolysis splits the starch so that each amylogen complex is divided into three similar portions, which can be termed protodextrin-residues II. These residues each consist of three glucose molecules. Since protodextrin II contains six glucose molecules, it must consist of two protodextrin-residues II, and its constitution can be schematically arranged as follows:

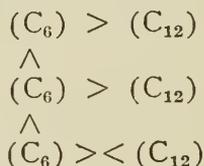


From this formula it is apparent that the molecule of this substance contains two intact γ bonds and two maltose groups con-

assumption of one di-carbonyl linking; or, there are nine mono-carbonyl bonds, which necessitates the assumption of a ring structure.

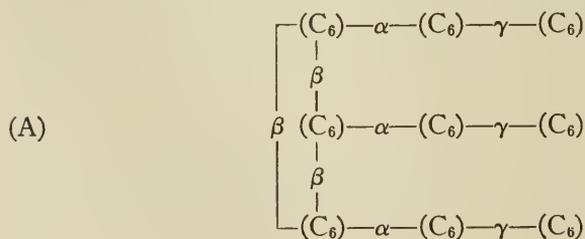
Let us consider the first possibility, for which a di-carbonyl group is assumed. It is evident that none of the γ bonds is a carbonyl (there is no di-carbonyl bond in maltose). It is also easy to see that none of the β bonds in the dextrin residue is di-carbonyl. Free protodextrin I reduces Fehling solution, which indicates that it contains a free carbonyl radical. At the two linkings between the three glucose molecules of this dextrin, only two carbonyl radicals are formed upon hydrolysis: these are mono-carbonyl.

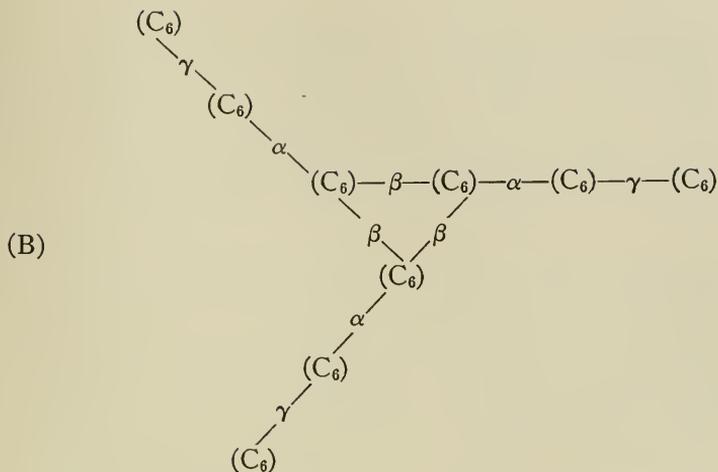
If a di-carbonyl linking were present in the amylogen, it could be only one of the α bonds. By use of the sign ($<$) to denote a carbonyl bond, and putting di-carbonyl bonds in the place of α linkings, the formula of amylogen may be written as follows:



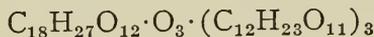
Under this scheme, hydrolysis would give no maltose; but, on the other hand, two different kinds of dextrin, one possessing no reducing power. This is not the case and, therefore, there is no di-carbonyl bond in the amylogen nucleus.

This conclusion naturally suggests the other possibility. The nature of the hydrolytic products of amylogen that have been studied would make it difficult to imagine an α or a γ linking in a ring structure. Such a structure, Synkiewski claims, may be easily made up of β bonds, and there are two possibilities:

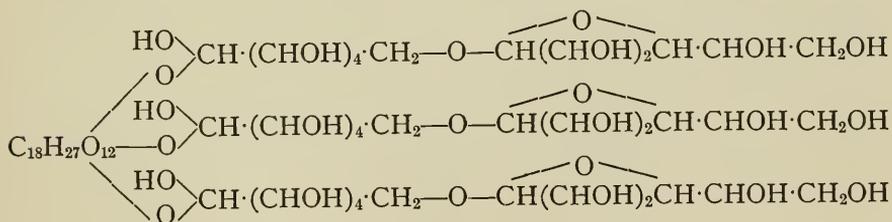




As shown before, the rational formula for the amylogen group is some multiple of



and by making use of Fischer's formula for maltose, Synkiewski writes the graphic formula for amylogen as follows:



Synkiewski suggested that this formula represents a half acetal of the ten-hydroxyl alcohol of maltose, which is analogous to the alcoholates of chloral; and that it might be named "tri-malto tri-glucosate," if the not impossible but as yet undiscovered triglucose sugar were known to exist.

In order to combine all facts, including a ring combination of the three glucose molecules, Synkiewski shows that the formula for the amylogen nucleus could be written in accord with the arrangement shown in Fig. 3, on p. 392.

The nature of the linkings between the amylogen groups in the starch molecule is deduced through the fact that, in the formation of soluble starch or amyloextrin, hydrolysis takes place without the formation of reducing compounds, from which it is concluded that a carbinol hydrolysis has taken place; hence the amylogens must be connected one to another in an ether-like or carbinol manner.

The next step was the construction of the constitutional formula for the starch molecule and incidentally to set limits by geometrical means for the possible sizes which the starch molecule may assume. The fact that after β -hydrolysis, molecules of only a single dextrin of two C_{18} -groups are obtained is proof that the carbinol bonds contained in these molecules, and the carbinol hydroxyls of the amylogen residue which bring about the union, are of equal value. These three hydroxyls must occupy similar places in the amylogen molecules. If we connect the middle points of these places we unconsciously form an equilateral triangle on each corner of which is located the dextrin carbinol bond.

Since the three dextrin-carbinol bonds of each amylogen must be equivalent to similar bonds of all other amylogens, we assume that this condition will find expression in the parallel position of these bonds in the starch molecule. The triangles, which we have assumed to be the configurations of the amylogens, must be arranged in the starch molecule so that every two neighboring groups will have similar, opposite, positions.

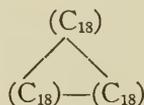
On the ground of the above argument, in the construction of the starch molecule, only so many amylogens take part as the number of equilateral triangles that can come together in a closed figure in which every two of them shall always have an adjacent angle and similar opposite positions. There are only four geometrical constructions, according to Synkiewski, which can accommodate these premises.

Place two triangles atop each other and we have two superimposed triangles. Place a triangle at each edge, and have all triangles possess a side in common, and we arrive at a tetrahedron. Again, by similar processes we may arrive at an octahedron; finally at an icosahedron. This hypothesis allows only four alternatives

to choose from, a molecule consisting of two, four, eight or twenty amylogen groups.

Synkiewski points out that in forming amylodextrin only the maltose carbinol bonds were dissolved, the dextrin-carbinol bonds remaining intact. These dextrin-carbinol bonds were also not attacked by malt at 78° C. By a β -hydrolysis, protodextrin II is formed, in which these linkings persist. By solution of the α -bonds, maltose is split off and dextrinose is left, in which the dextrin-carbinol bond remains. Dextrinose is also obtained from protodextrin I, which shows that this substance has the bond intact; and, since it comes from amylodextrin, it must have just as many dextrin groups as amylodextrin and therefore just as many as amylogen. From the molecular weight of protodextrin I it follows that it consists of four protodextrin I residues; therefore, the starch molecule is composed of four amylogen residues and its empirical formula must be $C_{216}H_{360}O_{180}$.

For the sake of simplicity, the amylogen residues are represented in triangular form:



and the starch molecule may then be represented on a plane surface as follows:

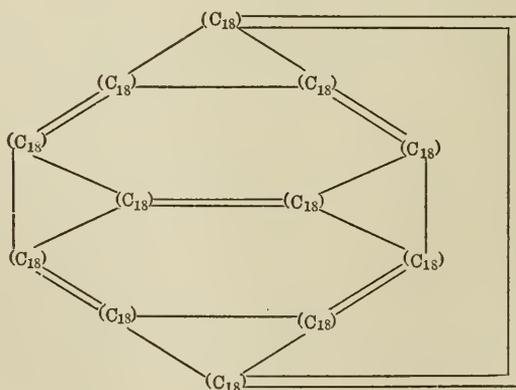


FIG. 4.

The elaborate work of Synkiewski ends with this hypothetical configuration for the starch molecule, the size of which $(C_6H_{10}O_5)_{36}$, is only about one sixth as large as that proposed by Brown and Morris, $(C_6H_{10}O_5)_{200}$. The experimental work of Brown and Morris is not nearly so extensively applied to the question at issue as that of Synkiewski, and on that account one might be inclined to give preference to Synkiewski's suggested molecule; but it must be borne in mind that Synkiewski's hypothesis rests entirely on two dextrans, protodextrin I and protodextrin II, the one formed by malt diastase in cold solution and the other in hot solution. He assumes that these dextrans of different molecular weight are different individuals and that both are present in the starch molecule simultaneously. This assumption is open to doubt, however, since malt diastase does not appear to have any different influence in hot and cold solutions other than that due to difference in rate of hydrolytic action.

Since Synkiewski's work appeared there have been several papers of minor import dealing with this same topic. During the same year there was published by Hale²³ a review of work previously reported about the constitution of starch; also an account of his own ideas and the results of a study of starch iodides. He constructed a molecular formula embodying all the good features of former workers. From his own work on starch iodide he concluded that the size of the starch molecule might be at least $(C_6H_{10}O_5)_{18}$, because he had obtained an iodide of starch containing 4.38 percent of iodine; expressing the formula $(C_6H_{10}O_5)_{18}I$. This formula does not merit much consideration because, with our present knowledge of colloidal chemistry, we are prone to regard the blue complex formed by starch and iodine as a special colloidal phenomenon.

In 1904, Kladiaschwili²⁴ treated starch with formic acid and obtained a mono-formyl derivative which seemed to indicate by the freezing-point-depression-method in chloracetic acid and phenol solutions the formula $(C_7H_{10}O_6)_6$. There is danger, however, that this derivative is a hydrolytic product of starch.

²³ Hale: *School of Mines Quarterly*, 1902, xxiv, p. 125.

²⁴ Kladiaschwili: *J. Russ. Phys. Chem. Soc.*, 1904, xxxvi, p. 905.

In 1905, Skraup,²⁵ working along similar lines, acetylated dextrans and starch derivatives under the influence of hydrochloric acid gas at -20° C. From a study of some of the chloracetyl derivatives he announced his belief that starch has a molecular weight of $(C_6H_{10}O_5)_{46}$ to $(C_6H_{10}O_5)_{50}$.

A method altogether different in principle from those preceding was published in 1909 by Wacker.²⁶ The procedure consisted in mixing the substance to be investigated with phenylhydrazine sulfonic acid and alkali, when a red color was formed, the depth of color depending upon the molecular weight. This method worked very well with carbohydrates and compounds having alcohol and aldehyde groups. Wacker's conclusion, however, that starch is made up of two substances, one $(C_6H_{10}O_5)_6$ and another "of a more cellulose character," $(C_6H_{10}O_5)_7$ to $(C_6H_{10}O_5)_8$, indicates that his method does not hold in the case of starch solutions.

It is very strange that many modern chemists, or chemists living in modern times, still seem to think that starch has a low molecular weight. The large amount of trustworthy experimental evidence which has been reported in the last fifteen years ought to dispel such a notion. Nevertheless, in a recent review, Frankforter²⁷ seriously considered such formulas as $(C_6H_{10}O_5)_2$, $(C_6H_{10}O_5)_4$, and $(C_6H_{10}O_5)_6$, giving his preference to the "hexapolymer." He cited Wacker's experiments, drawing from them the conclusion that "the starch molecule is at least of the size $(C_6H_{10}O_5)_6$ or probably greater." He included a graphical formula, made up of six glucose groups united by oxygen linkings in a continuous structure.

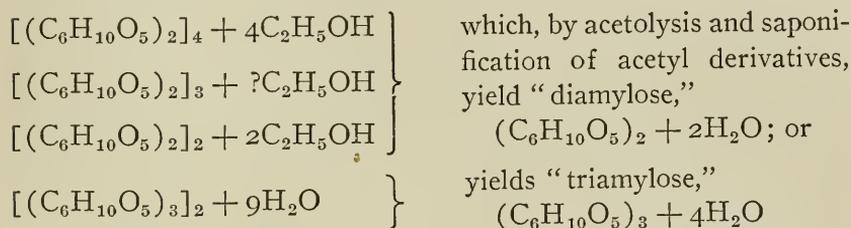
Such a simple structure seems quite impossible. Malt hydrolysis of starch results in the formation of maltose and of dextrin of great complexity. We feel confident that malt diastase as ordinarily used is a hydrolyzing and not a constructive agent. Where could the complex dextrinous substances come from if the starch molecule were a simple hexapolymer of glucose? Does not also the colloidal nature of starch in solution suggest a high molecular weight?

²⁵ Skraup: *Monatsh. Chem.*, 1905, xxvi, p. 1415.

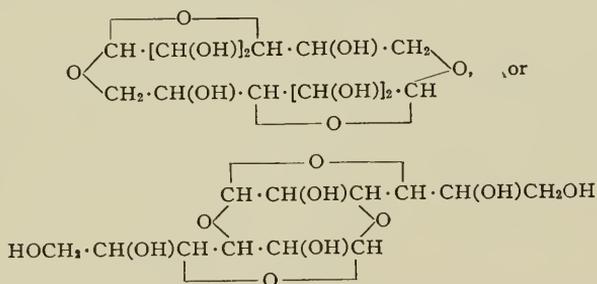
²⁶ Wacker: *Berichte*, 1909, xlii, p. 2695.

²⁷ Frankforter: *Proc. 8th Intern. Congr. Appl. Chem.*, 1912, viii, p. 133.

From 1912 to 1915 the work of Pringsheim²⁸ has helped to establish more firmly than ever the theory of the great molecular complexity of starch. By fermentation and hydrolysis of starch, under different conditions, he obtained several dextrans which were tetra- and hexa-polymers of the simple dextrin molecule and similar to those isolated by Schardinger:²⁹



From his experimental results he concludes that these dextrans or "amyloses" have a ring structure, such as



and that they indicate that starch itself, and the non-reducing dextrans which are not fermented by yeast, contain the ring which can be opened only by special ferments.

In the light of all the observed properties of starch, we are obliged to accept the hypothesis that the molecule of this substance is large and very complex. Further experimental work, perhaps from the synthetical standpoint, will doubtless throw much more light upon the limits of magnitude of the molecule.

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²⁸ Pringsheim: *Berichte*, 1913, xlv, p. 2533; 1915, xlvii, p. 2565. *Landw. Vers. Stat.*, 1914, lxxxiv, p. 267. *Naturwissenschaften*, 1915, iii, p. 95.

²⁹ Schardinger: *Zentralbl. f. Bacteriologie* (II Abt.), 1905, xiv, p. 772; 1907, xix, p. 161; 1909, xxii, p. 198; 1911, xxix, p. 188.

THE BEHAVIOR OF TARTARIC ACID AND THE TARTRATES IN THE ANIMAL ORGANISM

MAX KAHN

Introduction. In a recent paper by Carles,¹ attention was drawn to the important part played by tartaric acid in wine manufacture, and a prophesy was made that within ten years the consumption of tartaric acid would be increased ten-fold. Tartaric acid and tartrates are used extensively, not only in wines but also in baking powders, in medicated waters and in candies. Comparatively little experimental work has been done on the pharmacology of tartaric acid and tartrates. The importance of exact knowledge in these connections is evident.

Dextro-tartaric acid was isolated first by Scheele, in 1768, and obtained in crystalline form by Retzius, in 1770, who designated it *Sal essentielle tartari*. Its composition and salts were studied by Gay-Lussac, Berzelius, and others; its optical properties, especially, by Biot (1815) and Pasteur (1841).² Four optical isomers are known: *dextro-*, *levo-*, *para-* (racemic), and *meso-*tartaric acids.

Tartaric acid is one of the most widely distributed acids in the plant kingdom. It is present in the free state, or in the form of salts, in grapes, mountain-ash berries, tamarinds,³ tomatoes,⁴ cucumbers, potatoes, black pepper, pineapple and in leguminous plants.⁵ It has also been found in grape leaves,⁶ senna leaves,⁷ liverwort,⁸ ferns, beet juice, and fungi.⁹

¹ Carles: *Répert pharm.*, 1913, xxiv, p. 387.

² Hare, Caspari and Rusby: *National Dispensatory*, 1905, p. 90.

³ Adam: *Zeit. d. österr. Apoth.-Vereins*, 1905, xliiii, p. 797.

⁴ Albahary: *Compt. rend.*, 1907, cxlv, p. 137.

⁵ Müller: *Arch. d. Pharmaz.*, 1883, ccxxi, p. 42; Heckel and Schlagdenhauffen: *Jour. d. pharm. et d. chim.*, 1889, xix, p. 11.

⁶ Piti: *Ber. d. d. chem. Ges.*, 1873, vi, p. 1313.

⁷ Wallis: *Pharmaceut. Jour.*, 1912, xxxv, p. 644.

⁸ Zopf: *Die Pilze*, Breslau, 1890.

⁹ Fritsch: *Arch. d. Pharmaz.*, 1889, ccxxvii, p. 193.

Behavior toward bacteria and fungi. Tartaric acid is produced, in the fermentation of fruit and grape juice, by the *Apiculatus* yeast, in which process carbohydrates are oxidized to tartaric acid. Calcium tartrate may be fermented, by the *Bacillus tartaricus*, to acetic acid, succinic acid, carbon dioxid and hydrogen.¹⁰ In the presence of ammonium nitrate, tartaric acid is fermented to propionic acid, acetic acid and carbon dioxid.¹¹

Certain yeasts use tartaric acid as food, absorbing it in their growth.¹² Pasteur¹³ found that *Penicillium glaucum* so affects *p*-tartaric acid (racemic) that it is changed to *l*-tartaric acid. Yeast ferments *d*-tartaric acid much more easily than the *l* form. The final carbonaceous product in the catabolism of tartaric acid by yeast is carbon dioxid.¹⁴ The peculiar affinity of yeast for the *d*-acid is quite significant, as will be seen below, where the metabolism of the various tartaric acids in the animal body is considered.

The mycoderms have no effect on tartaric acid.¹⁵ According to Waterman,¹⁶ *l*- and *d*-tartaric acids may be utilized by *Aspergillus niger* as a source of carbon. Racemic acid is scarcely attacked by this organism but, after a prolonged period, mutation occurs. Neuberg and Czapski^{16a} found that a concentration of 0.45 *M* of *d*-tartaric acid retards the fermentation of glucose.

General observations on toxicity. Certain of the earlier writers on the toxicology of tartaric acid considered this substance entirely non-poisonous. Christison¹⁷ concluded, from the results of an experiment by him and Coindet, in which they administered *per os* to a cat 3.75 gm. of tartaric acid dissolved in water, that this acid is wholly non-toxic. He also cited the experience of Dr. Sibbald, of Edinburgh, who accidentally ingested 22.5 gm. of the acid without suffering any ill effects. Wibmer stated that tartaric

¹⁰ Pasteur: *Compt. rend.*, 1858, xlvi, p. 615; 1863, lvi, p. 416. Grimbert and Fiquet: *Jour. de pharm. et d. chim.*, 1898, vii, p. 97.

¹¹ König: *Ber. d. d. chem. Ges.*, 1881, xiv, p. 211.

¹² Bail: *Centr. f. Bakter. u. Parasitenk.*, 1902, viii, p. 567.

¹³ Pasteur: *Compt. rend.*, 1858, xlvi, p. 615.

¹⁴ Karczag: *Biochem. Zeit.*, 1912, xxxviii, p. 516.

¹⁵ Meissner: *Ber. d. königl. Württemb. Weinbau-Versuch.*, 1904, p. 72.

¹⁶ Watermann: *Chem. Zentralbl.*, 1914, i, p. 485.

^{16a} Neuberg and Czapski: *Biochem. Zeit.*, 1914, lxxvii, p. 51.

¹⁷ Christison: *Abhandlung über die Gifte*, 1831, p. 212.

acid is injurious to the alimentary canal, hindering digestion, being more toxic, in these relations, than citric acid; also more diuretic than the latter acid.¹⁸

Soon after the appearance of Wibmer's publication, certain cases of fatal poisoning were reported. In Watkins' case,¹⁹ a young man, 24 years of age, took 30 gm. of tartaric acid in mistake for bitter salts. He suffered from violent pains and debility, and died on the ninth day. Devergie²⁰ described another case of fatal poisoning with tartaric acid. Belloc²¹ wrote of a case of poisoning by Rochelle salt.

These cases caused a change in the attitude of toxicologists toward the tartaric acids. Orfila²² regarded them as very irritating and therefore considered them toxic. He also treated a patient who, when intoxicated, took 120 gm. of potassium tartrate and died on the fourth day afterward. Van Hasselt²³ regarded tartaric acid as toxic only in large doses. He considered it neither more nor less toxic than citric acid. He described its effects as resembling those of oxalic acid poisoning, differing only in a slower rate in the initiation of the symptoms. Hermann²⁴ expressed the opinion that tartaric acid is toxic only in the free state; when in union with a base, its toxicity is that of the base to which it is attached. Jaksch²⁵ stated that tartaric acid causes gastro-intestinal catarrh, with cramps and diarrhea. He did not mention the dose.

Trevithick²⁶ reported the case of a woman, 67 years old, who took 12 gm. of tartaric acid by mistake. She suffered pains all over the body, vomiting and diarrhea ensuing. On the fourth day delirium developed, temperature became subnormal, pulse very weak. Death occurred on the seventh day.

¹⁸ Wibmer: *Wirkung der Arzneimittel und Gifte*, Munich, 1842, v, p. 319.

¹⁹ Watkins: *Jour. d. chim. med.*, 1845, i, p. 220.

²⁰ Devergie: *Ann. d'Hyg.*, 1845, xi, p. 432.

²¹ Belloc: *Cours de med. leg.*, 139, cited by Taylor; *Die Gifte in gerichtlichen Medizin*, Cologne, 1863, ii, p. 131.

²² Orfila: *Lehr. d. Toxikologie*, Braunschweig, 1852, i, p. 154.

²³ Van Hasselt: *Allgemeine Giftlehre und die Gifte des Pflanzenreichs*, Braunschweig, 1862, i, p. 532.

²⁴ Hermann: *Lehrb. d. exp. Toxikol.*, Berlin, 1874, p. 153.

²⁵ Jaksch: *Die Vergiftungen*, Vienna, 1897, p. 43.

²⁶ Trevithick: *Brit. Med. Jour.*, June 24, 1903.

Experimental toxicology. Mitscherlich²⁷ was one of the first to conduct a series of experiments to determine the toxicity of tartaric acid. In his work on rabbits and cats, he found that 10 gm. of the acid were necessary to kill a rabbit, when the substance was administered *per os*. In a cat, 5 gm. failed to induce symptoms. He also found that rabbits are more susceptible to citric acid than to tartaric, 5 gm. of the former being sufficient to cause death.

After painting the skin of a frog with dilute citric or tartaric acid, Goltz and Bobrick²⁸ observed very marked slowing of the heart, with final stoppage.

In 1893, Chabrié,²⁹ investigating the differences in toxicity of the various stereoisomeric tartaric acids, found that *l*-tartaric acid is the most toxic, whereas *d*-tartaric acid is only half as toxic. He determined the lethal dose for rabbits in the following way: Certain quantities of the acids were necessary to kill rabbits of the same weight in a certain time. There was a definite ratio between these doses, which he expressed by the function $\frac{I}{X}$. He suggested the following formula:

$$\frac{I}{X} = \frac{p \cdot 1000}{P} \cdot T$$

where *p* is the dose used, *P* the weight of the animal in gm., and *T* the time in minutes. From his experiments he found,

$$\begin{aligned} X(l) &= 0.031; \\ X(d) &= 0.014; \\ X(r) &= 0.008; \\ X(m) &= 0.006. \end{aligned}$$

Attention has already been called to the difference in the behavior of these stereoisomeres in yeast fermentation. Chabrié also reported that *p*-tartaric acid (racemic) is only one fourth as toxic as the *d*-acid, and that the *m*-acid is wholly non-toxic.

²⁷ Mitscherlich: De acidi acetici, oxalici, tartarici, formici et boracici effectu in animalibus observato, Berlin, 1845, p. 27.

²⁸ Goltz and Bobrick: *Königsberg med. Jahrb.*, 1863, iv, p. 95.

²⁹ Chabrié: *Compt. rend.*, 1893, cxvi, p. 1410.

Chio,³⁰ accepting Chabrié's conclusions as correct, explained the different toxicologic behaviors as follows: The four stereoisomeric acids, which are different in their toxic behavior, modify *in vitro* the concentration of the H ions in the *same* manner; in the guinea pig they produce a very mild reaction. They fix calcium with a *different* activity in solutions of calcium carbonate in water (saturated with carbon dioxid), in beef serum, or in dog serum. The degrees of their toxicity are not in accord with the slight variation in concentration of H ions which is effected by them in the circulating blood, but *depend on the extent to which they abstract calcium from the tissues of the organism.*³¹

Vietinghoff-Scheel³² found that after subcutaneous or intravenous administration of tartrates into frogs, mice and rabbits, neutral sodium citrate is more toxic than neutral sodium tartrate, as is the case when the acids are administered in the free state. In order to produce initial toxic symptoms in a rabbit he was obliged to inject 1 gm. of the tartrate, whereas 0.2 gm. of the citrate was sufficient to induce poisonous effects.

Steinfeld³³ made several experiments on frogs, and observed that the injection of 0.06 to 0.08 gm. of tartaric acid had no effect; 0.3 gm. induced paralysis of the nerves and muscles and death. A cat that received 2 gm. of the acid *per os* showed no toxic symptoms.

Salant and Smith³⁴ tested the toxicity of the sodium salts of *d*- and *l*-tartaric acid on frogs and rabbits. These isomeres were found equally toxic in these animals, thus contradicting the earlier work of Chabrié on the subject. In the experiments on rabbits, the diet proved to be an important factor in the determinations of tolerance for this substance. Animals which were fed on oats, or oats and cabbage, succumbed to a dose of 0.4 gm. of the salt per k., when given by subcutaneous injection. Suppression of urine was usually observed on the first day and death usually occurred

³⁰ Chio: *Arch. intern. pharmacodyn.*, 1913, xxii, p. 473.

³¹ See *Chemical Abstracts*, 1913, vii, p. 2622.

³² Vietinghoff-Scheel: *Arch. intern. de pharmacodyn. et de therapie*, 1902, x, p. 145.

³³ Steinfeld: cited by Vietinghoff-Scheel (footnote 32).

³⁴ Salant and Smith: *Proc. Soc. Exp. Biol. and Med.*, 1913, x, p. 170.

in 6 to 7 days. During fasting, slightly smaller doses were fatal to some rabbits. Resistance was considerably increased when the diet was changed to carrots. Animals on a carrot diet stood 1.0 gm. per k. by subcutaneous injection, but 1.2 to 1.5 gm. per k. was toxic. A moderate degree of tolerance for tartrates was induced in animals fed on oats and cabbage. By gradually increasing the dose, a large proportion (6 out of 9) of the rabbits survived 0.8 gm. per k., which is twice the fatal dose. Rabbits which were receiving carrots did not acquire tolerance for tartrates. Sodium tartrate was much less toxic when given by mouth. The minimum fatal dose was 5 gm. per k. Cats are less susceptible to tartrates than rabbits, as has long been known. Subcutaneous injection of 1 gm. per k. into cats induced slight diarrhea in some individuals, and had no effect whatever in others. One and one-half gm. per k. proved fatal to one cat, but was without action in another. Of four cats that received 2 gm. per k., three died and one survived. When sodium tartrate was given by mouth, vomiting frequently occurred. In one case, however, when 10 gm. per k. were fed, diarrhea was the only effect observed.

Upon infusoria and algae, tartaric acid acts very destructively. Bokorny³⁵ noticed that tartaric acid, at a concentration of 0.05 percent, kills *Spirogyra* and *Spharoplea* in 34 min; 0.01 percent was fatal in several days. Neutral salts of this acid have no toxic effect on these organisms. Vietinghoff-Scheel came to the same conclusion. He found that concentrations less than 1 percent of the neutral salts were without effect on infusoria.

Experimental pharmacology. Several observers have investigated the changes in the *blood* due to the administration of tartaric acid or its salts. Freudberg³⁶ found that 5 to 10 gm. of the acid administered *per os* to dogs, caused reduction in the alkalinity of the blood, amounting to 16 percent. Wallace and Cushny³⁷ observed that sodium tartrate is absorbed at about the same rate as the sulfate.

Vietinghoff-Scheel did not observe any effect of neutral sodium

³⁵ Bokorny: *Pflüger's Arch.*, 1896, lxiv, p. 278.

³⁶ Freudberg: *Virchow's Arch.*, 1891, cxxv, p. 566.

³⁷ Wallace and Cushny: *Amer. Jour. Physiol.*, 1898, i, p. 411.

tartrate on blood *coagulation*. Buglia and Karczag,³⁸ however, noted an inhibiting effect. Vietinghoff-Scheel found that small quantities of sodium tartrate inhibited the coagulation of casein by rennin, comparatively large amounts preventing it. These results were ascribed to abstraction of calcium from the blood or milk by the tartrate. Chiari³⁹ found that 10 c.c. of 5 percent sol. of sodium tartrate, administered *per os*, caused perceptible reduction in the amount of soluble calcium in the intestines, the tartrate precipitating much of the calcium, which passed into the feces.

Karczag⁴⁰ injected, intravenously into dogs, 0.2 gm. each of the various tartaric acids. He found that *d*-tartaric acid caused transitory stimulation of the *vagus centre*, whereas the *l*-acid induced more prolonged stimulation. The *p*- (racemic) and *m*-acids were intermediate in their effects between the *d*- and *l*-acids. On perfusing an isolated turtle-heart with *n*/100 sol., the acids caused decrease in the force of the systole and loss of tone. The *m*-acid was less toxic than the other acids, on the ventricle. Both Sakai⁴¹ and Gros⁴² also reported depression of *isolated frog hearts* perfused with *n*/100 sol. of tartrates.

Salant and Hecht⁴³ found that perfusion of *isolated hearts* of dogs, cats, and frogs, with sodium tartrate, caused depression. "Thus, perfusion of the cat's heart with solutions of *n*/10, *n*/20, *n*/40 and *n*/100 sodium tartrate, in defibrinated blood diluted with Locke sol., or in Locke sol. alone, was followed by diminished activity of the heart, which became more marked as the concentration of sodium tartrate was increased. It may be remarked, however, that the action did not vary in the same ratio as the concentration. The effect of various dilutions was even better exemplified in experiments on frog hearts, for which very dilute sol. were employed. When *n*/300 sodium tartrate sol. was perfused for 30 to 60 seconds, a slight cardiac depression was observed, the systole

³⁸ Buglia and Karczag: *Rendiconti d. r. Acc. dei Lincei Roma*, 1909, xviii, p. 474.

³⁹ Chiari: *Arch. f. exp. Path. u. Pharmacol.*, 1910, lxiii, p. 434.

⁴⁰ Karczag: *Zeit. f. Biol.*, 1910, liii, p. 218.

⁴¹ Sakai: *Zeit. f. Biol.*, 1914, lxiv, p. 1.

⁴² Gros: *Arch. f. exp. Path. u. Pharm.*, 1913, lxxi, p. 395.

⁴³ Salant and Hecht: *Amer. Jour. Physiol.*, 1915, xxxvi, p. 132.

alone being decreased in some experiments, in others the decrease affected the diastole as well as the systole. This may be regarded as the minimum concentration which can produce any effect, since a $n/400$ sol. proved to be without any action. . . . A very noticeable difference in the action of sodium tartrate was also obtained by varying the perfusion time." They also found that the citrate was more depressant than the tartrate.

Brion⁴⁴ found that the various stereoisomeres of tartaric acid are *differently catabolized* in the animal organism. He observed, as Chabrié did before, that the four tartaric acids are differently oxidized in the body. The *l*- and *m*-acids are oxidized more rapidly and more completely than the *d*-acid and still more so than the inactive *p*-acid (racemic). However, Brion's results have not been corroborated. Neuberg and Saneyoschi⁴⁵ found that, in the same dog, there is a difference in the amount of oxidation of a given tartaric acid at various times. They did not notice any difference in the oxidation of the various tartaric acids. They also found that administration of *p*-tartaric (racemic) acid resulted in the excretion of this inactive acid in the urine. Underhill, Wells and Goldschmidt,⁴⁶ found that sodium tartrate administered subcutaneously to rabbits (dose 0.5 to 0.765 gm.) could not be recovered in the urine, and concluded that the disintegrative influence of the salt upon the convoluted tubules is sufficient to account for the failure of the salt to appear in the urine.

The amount of tartaric acid that may be *oxidized in the body* has been variously estimated. Freudberg found that the greater portion is oxidized. Vietinghoff-Scheel stated that the acid "is very easily burnt" in the animal organism. Both Piotrowsky⁴⁷ and Magawly⁴⁸ found that tartaric acid is almost completely consumed when given *per os*, only slight traces of alkali or calcium salt being excreted in the urine. Pohl⁴⁹ administered sodium tartrate *per os* to dogs and rabbits, and found that only about 33 per-

⁴⁴ Brion: *Zeit. f. physiol. Chem.*, 1898, xxxv, p. 283.

⁴⁵ Neuberg and Saneyoschi: *Biochem. Zeit.*, 1911, xxxvi, p. 32.

⁴⁶ Underhill, Wells and Goldschmidt: *Jour. Exp. Med.*, 1913, xviii, p. 317.

⁴⁷ Piotrowsky: Dissertation, Dorpat, 1856.

⁴⁸ Magawly: Dissertation, Dorpat, 1856; cited by Vietinghoff-Scheel.

⁴⁹ Pohl: *Arch. exp. Path.*, 1896, xxxvii, p. 424.

cent was oxidized in the organism, the rest having been excreted unchanged in the urine.

Baumgarten⁵⁰ observed that tartaric acid is oxidized as well by *diabetic animals* as by normal ones. He fed normal and pancreatectomized dogs with tartaric acid and observed equal amounts of oxidation.

It was reported by Baer and Blum⁵¹ that subcutaneous administration of 8.8 gm. of sodium tartrate, to *phlorhizinized dogs* weighing about 12 k., caused great diminution in the urinary output of nitrogen and glucose. Underhill⁵² substantiated these findings. His experience shows that sodium tartrate, subcutaneously administered to phlorhizinized rabbits and dogs, induces disintegrative changes in the kidney tubuli sufficient to account for the diminished elimination of urinary nitrogen and glucose, as observed by Baer and Blum. Under strictly comparable experimental conditions similar results were obtained by Underhill in normal animals, "thus demonstrating that sodium tartrate acts specifically in this direction and that phlorhizin contributes little or nothing to the detrimental influence under discussion."

In a paper on the *experimental nephritis* induced by salts of tartaric acid, Pearce and Ringer⁵³ found that the administration of 10 to 15 gm. of Rochelle salt to dogs (*per os*, intraperitoneally, or subcutaneously) caused severe renal lesions, with the excretion in the urine of casts and albumin. Anuria occurred in some cases. The histological changes in the kidneys were necrosis of the convoluted tubules, with fatty changes in the loops of Henle and sometimes also in the collecting tubules. The mode of administration did not influence the character of the renal lesions. When tartrates were given by mouth, diarrhea was induced, which tended to cause rapid removal of the salt from the intestine, and thus probably prevented the severer types of renal lesions by reducing the amount of tartrate absorbed. In 1903, MacCallum⁵⁴ stated that the tar-

⁵⁰ Baumgarten: *Zeit. f. exp. Path. u. Ther.*, 1905, ii, p. 53.

⁵¹ Baer and Blum: *Hofmeister's Beitr.*, 1907, x, p. 80; *ibid.*, 1908, xi, p. 102; *Arch. f. exp. Path. u. Pharm.*, 1911, lxv, p. 1.

⁵² Underhill: *Jour. Biol. Chem.*, 1912, xii, p. 115.

⁵³ Pearce and Ringer: *Jour. Med. Res.*, 1913, xxix, p. 57.

⁵⁴ MacCallum: *Amer. Jour. Physiol.*, 1903, x, p. 101.

trates may be administered subcutaneously, with no bad results. He did not state the doses to which he referred, but he classed the tartrates with the sulfates and the citrates. Post⁵⁵ found that Rochelle salt, administered in *ordinary* doses by mouth to human beings, did not cause albuminuria or cylinduria, or aggravate an existing nephritis.

Connio,⁵⁶ in his experiments on dogs and rabbits, showed that the intravenous administration of tartaric acid induced marked albuminuria, nephritis and death. The nephritis produced was essentially glomerular, though the tubules were also involved. Given *per os* it induced vomiting and finally nephritis and death.⁵⁷

Underhill and his coworkers⁵⁸ have recently added much to our knowledge of the pathological effects of tartrates. In their experiments on rabbits and dogs they found that there is no strict relation between the dose of tartrate and the extent of damage inflicted. Histological study of the affected kidney tissue revealed that it was the epithelium of the convoluted tubules that was mostly involved, and to a less extent the loops of Henle. The glomerulus and the interstitial tissue remained intact. Neither the liver nor the adrenals showed any ill effects of the tartrate administration. The most effective way of administering the tartrates was found to be subcutaneously (in doses of 1.5 to 2.5 gm., to rabbits). Undernutrition increased susceptibility to the influences that caused renal lesions. It was also observed that the kidneys in tartrate nephritis lost the power of excreting urea injected intravenously, but the power of excreting chlorids so administered remained unimpaired.

Dakin⁵⁹ investigated the coefficient of *intestinal absorption* of the various tartaric acids. He found that all of the acids were absorbed equally, no selective absorption having been noticed.

In *liver perfusion* experiments, Ohta⁶⁰ found that, upon addition of 6 gm. of either *d*- or *p*-tartaric (racemic) acid to the perfusing blood, acetone was formed in the liver. The perfusion of succinic acid was not followed by this result.

⁵⁵ Post: *Jour. Amer. Med. Assoc.*, 1913, lxii, p. 592.

⁵⁶ Connio: *Arch. di antropol. crimin.*, 1911, xxxii, p. 438.

⁵⁷ Connio: *Path. riv. quindicin. Geneva*, 1910, iii, p. 428.

⁵⁸ Underhill, Wells and Goldschmidt: *Jour. Exp. Med.*, 1913, xviii, p. 322.

⁵⁹ Dakin: *Jour. Biol. Chem.*, 1908, iv, p. 437.

⁶⁰ Ohta: *Biochem. Zeit.*, 1912, xlv, p. 167.

Dr. William Salant⁶¹ and his coworkers have made a special and interesting study of the influence of sodium tartrate on the *circulation*: "Experiments with various concentrations of sodium tartrate were made on dogs. When the tartrate solution was injected into an animal under *chloretone anesthesia* the following results were obtained: The amplitude of cardiac pulsation, as shown by the Cushny myocardiograph, was decreased even when dilute solutions were employed, the systole being more affected than the diastole. There was also moderate slowing of the heart. Blood-pressure was not affected to any appreciable extent. The volume of the kidney, as shown by the oncometer, was only slightly increased. When *ether* or *morphine anesthesia* was used, the cardiac effects were not quite so uniform. In deep *ether anesthesia* cardiac amplitude and rate, after the injection of sodium tartrate, were the same as under *chloretone anesthesia*. When anesthesia was lighter, sodium tartrate was without effect on the rate or amplitude in some experiments; in others, the rate was increased by 15-25 percent, amplitude also being distinctly increased. A rise of blood-pressure accompanied injection of sodium tartrate; 0.1 to 0.4 gm. per k. producing a rise of 10 to 25 percent. In some experiments blood-pressure rose 40 to 50 percent. Recovery followed invariably. In *curarized animals* the action of sodium tartrate was more marked; 3-4 c.c. per k. of 2.5 percent sodium tartrate causing a rise of blood-pressure varying between 45 and 200 percent, which was also the case after the injection of 10 percent sodium tartrate sol. Successive injections made at short intervals produced the same effect, thus showing absence of accumulation. When the concentration was increased 20 percent, the action was reversed, and a distinct fall in blood-pressure was observed. The volume of the kidney was markedly increased. This was out of any proportion to the rise of blood-pressure and was very constant. The increase was simultaneous with the rise in blood-pressure, but recovery was much slower. Microscopic examination of the kidneys of rabbits that died as a result of the administration of sodium tartrate showed very marked congestion."

⁶¹ Private communication to the author. See also Salant and Hecht: *Amer. Jour. of Physiol.*, 1915, xxxvi, p. 126.

Dr. Salant has also observed, in some of his tartrate experiments, that "when both vagi were cut the blood-pressure rose slightly, while the cardiac action was hardly affected."

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DOCTORATES IN BIOLOGICAL CHEMISTRY

Conferred by American Universities, 1914-'15

The names of recent recipients of the Ph.D. degree in biochemical science, with the subjects of the dissertations, are arranged below in university groups.

Brown University.—*Ralph Gibney Hurlin*: Histogenesis and distribution of the connective-tissue pigmentation of the silky fowl.—*Benjamin Samuel Levine*: Removal of natural impurities of cotton cloth by action of bacteria.—*Courtland Sawin Mudge*: Effect of sterilization on sugars in culture media.—*George Hathorn Smith*: Parenteral digestion of bacterial protein.—*Albert Whitman Sweet*: Sanitary survey of the Seekonk River.

Columbia University.—*Arthur Donaldson Emmett*: Metabolism studies of fatigue, rest and recuperation.—*Frederick Grosvenor Goodridge*: Biochemical studies of mercaptan.—*Edward Gray Griffin*: Inosite and pinite, and some of their derivatives.—*Mildred Albro Hoge*: Influence of temperature on the development of a Mendelian character.—*Israel Jacob Kligler*: Biochemical studies and differentiation of oral bacteria, with special reference to dental caries.—*Dora Estelle Neun*: Examination of certain methods for the study of proteolytic action.—*Percy Withers Punnett*: Study of the products of the action of different amylases.—*Arthur Percival Tanberg*: Experiments on the amylase of *Aspergillus oryzae*.—*Arthur Waldorf Spittell Thomas*: Influence of certain acids and salts upon the activity of malt amylase.

Cornell University.—*Millard Alschuler Klein*: Studies in the drying of soils.—*Leonard Amby Maynard*: Fixation of nitrogen by sweet clover.—*James Kemp Plummer*: Effect of oxygen and carbon dioxid on nitrification and ammonification in soils.—*William Jacob Robbins*: Digestion of starch by *Penicillium* (*Camembertii*).—*James Kenneth Wilson*: Physiological studies of *Bacillus radicola* of soy bean (*Sojus max* Piper) and of factors influencing nodule production.

Harvard University.—*Thorne Martin Carpenter*: Comparison of methods for determining the respiratory exchange in man.—*Frederick Simonds Hammett*: Uric acid in tissues.—*Guilford Bevil Reed*: Studies in plant oxidases.

Johns Hopkins University.—*Walter Hatheral Coolidge*: Osmotic-pressure measurements of glucose solutions at 10° and 20°.—*James Eugene Levering Holmes*: Difference in chemical behavior of free and combined water, as illustrated by the saponification of esters.—*Forman Taylor McLean*: Preliminary study of climatic conditions in Maryland, as related to the growth of soy-bean seedlings.—*Amos Sentman Musselman*: Osmotic-pressure measurements of glucose solutions at 30°, 40°, 50° and 60°.—*Lyde Stuart Pratt*: Esterification of benzoic acid by mercaptans.—*John Wesley Shive*: Study of physiological balance in nutrient media resulting in a simplified culture-solution for plants.

Northwestern University.—*Siegel Buckborough*: Structure of maltose and its oxidation products with alkaline peroxid of hydrogen.

University of California.—*Oscar Leo Brauer*: Rate of conversion of cinchonin into cinchotoxin.—*Richard Morris Holman*: Orientation of terrestrial roots, with particular reference to the medium in which they are grown.—*Charles Walter Porter*: Temperature coefficients and the effects of acids, bases and neutral salts in reaction velocities of the triphenylmethane dyes.

University of Chicago.—*Joseph Stuart Caldwell*: Study of the effects of certain antagonistic solutions upon the growth of *Zea mays*.—*Walter Lee Gaines*: Contribution to the physiology of lactation.—*Edwin Frederick Hirsch*: Experimental study of the influence of iodine and iodides on the absorption of granulation tissue and fat-free tubercle bacilli.—*Charles Edwin King*: Origin of the diastases of blood and lymph.—*Julian Herman Lewis*: Absorption of substances injected subcutaneously, and the inhibitory action of heterologous protein-mixtures on anaphylaxis.—*Agnes Fay Morgan*: (I) Viscosities of various methyl and ethyl imido-benzoates, and of the sodium salts of para- and meta-nitrobenzoylchloroamides in moderately concentrated aqueous solutions; (II) Molecular rearrangement of some triaryl methylchloroamines.—*George Burton Rigg*: Decay and soil toxins.—*Clare Christman Todd*: Action of alkaline hydrogen peroxid on *d*-galactose.

University of Illinois.—*William Leonidas Burlison*: Availability of mineral phosphates for plant nutrition.—*Harry Peach Corson*: Manganese in water supplies.—*Wallace Macfarlane*: Solubility of lime carbonates in relation to their endurance in soils.—*Harold Hanson Mitchell*: Feeding experiments on the substitution of proteins by definite mixtures of isolated amino-acids.—*Fred Weaver Muncie*: Effect of large applications of commercial fertilizers upon carnations.—*Morris Miller Wells*: Relations of fishes to ions in their natural environment: (I) Reactions and resistance to acidity, alkalinity, and neutrality; (II) Reaction and resistance to salts.—*Frank Archibald Wyatt*: Influence of calcium and magnesium compounds on plant growth.

University of Michigan.—*George Herbert Coons*: Study of the factors involved in the growth and pycnidia formation of *Plenodromus fuscomaculans*.

University of Minnesota.—*Morris Joslin Blish*: Chemical constitution of wheat proteins and their relation to baking "strength" in flour.

University of Wisconsin.—*John Nicholas Lowe*: Action of chemical stimuli on the chromatophores of brook trout, *Salvelinus fontinalis mitchill*.—*Charles August Mann*: Chemistry of San Palmetto berries.—*Howard Edward Pulling*: Movement of water in aërotid soils.

Washington University.—*Alva Raymond Davis*: Enzyme action in the marine algæ.—*Joseph Charles Gilman*: Cabbage yellows and the relation of temperature to its occurrence.—*Melvin Clarence Merrill*: Electrolytic determination of exosmosis from the roots of plants subjected to the action of various agents.

Yale University.—*Joseph Sumner Bates*: Synthesis of dipeptid-hydantoins, together with a short study of Michigan hard-wood tar.—*Emil Jacob Baumann*: Question of fat absorption from the stomach.—*Isaac Faust Harris*: Chemical and physiological studies of the castor bean and soy bean.—*Byron Murray Hendrix*: Studies in the physiological action of some protein derivatives.—*Edward Frederick Kohmann*: Constitution of mono- and di-nitrotyrosin, and the xanthoproteic and Millon reactions.—*Walter Moody Scott*: Hydroxyl derivatives of phenylalanin, and their biochemical inter-

est.—*Raymond Louis Stehle*: Rôle of the digestive glands in the excretion of endogenous uric acid.—*Richard Wrenshall*: Synthesis of α -amino- δ -phenylvalerianic acid.

Universities that conferred Ph.D. degrees in the natural and exact sciences, but at which there were no biochemical candidates are named below:

Catholic University	Stanford University
Clark University	Tulane University
George Washington Univ.	University of Iowa
Indiana University	University of Missouri
Mass. Inst. of Technology	University of Nebraska
New York University	Univ. of North Carolina
Ohio State University	Univ. of Pennsylvania
Princeton University	University of Pittsburgh

Number of awards of the Ph.D. degree, by American universities, to biochemical candidates: 1912, 1913, 1914 and 1915

	Men and Women				Total	Women				Total
	1912	1913	1914	1915		1912	1913	1914	1915	
Brown University	1	0	1	5	7	0	0	0	0	0
Clark University	0	0	1	0	1	0	0	1	0	1
Columbia University	11	7	7	9	34	1	0	1	2	4
Cornell University	5	2	4	5	16	0	1	1	0	2
Harvard University	1	1	4	3	9	0	0	0	0	0
Indiana University	0	0	1	0	1	0	0	0	0	0
Johns Hopkins University ..	1	1	2	6	10	0	0	1	0	1
New York University	0	0	1	0	1	0	0	0	0	0
Northwestern University ...	0	0	0	1	1	0	0	0	0	0
University of California ...	5	0	2	3	10	1	0	1	0	2
University of Chicago	8	4	3	8	23	1	0	0	1	2
University of Illinois	5	0	3	7	15	0	0	0	0	0
University of Michigan	2	0	1	1	4	0	0	0	0	0
University of Minnesota ...	0	0	1	1	2	0	0	0	0	0
University of Missouri	0	1	0	0	1	0	0	0	0	0
University of Pennsylvania..	0	0	1	0	1	0	0	0	0	0
University of Wisconsin ...	4	3	4	3	14	0	1	0	0	1
Washington University	0	2	0	3	5	0	0	0	0	0
Yale University	6	4	6	8	24	1	1	0	0	2
Total number of awards										
of degrees	49	25	42	63	179	4	3	5	3	15
Number of universities										
awarding the degree	11	9	16	14	—	4	3	5	2	—

THE BIOCHEMICAL SOCIETY, ENGLAND.

PROCEEDINGS REPORTED BY R. H. A. PLIMMER, SECRETARY

I. HONORARY SECRETARY'S ANNOUNCEMENT OF THE SCIENTIFIC PURPOSE AND SCOPE OF THE MEETING ON MAY 5, 1915¹

The Meeting is to be devoted to a discussion of "Methods adopted in the estimation of the nitrogenous constituents of extracts derived from albuminous substances such as meat extracts and similar products, with special reference to the interpretation of the results."

The following scheme, which represents the chemical methods commonly made use of in the examination of nitrogenous extracts, is intended to serve as the basis of discussion:

Meat Fibre and Coagulable Albumenoids. Ten (10) grammes of the extract are dissolved in 100 c.c. of cold water. Five (5) drops of acetic acid are added and the solution brought to the boil and allowed to boil gently for 5 minutes. It is then filtered and the precipitate is washed with warm water, and the nitrogen determined by Kjeldahl's method in the ordinary way. $N \times 6.25 =$ Meat fibre and coagulable albumenoids.

Albumoses and Peptones. The filtrate from the meat fibre and coagulable albumenoids is made up to a definite volume and an amount corresponding with 5 grammes of the original extract pipetted out. To this is added a few drops of hydrochloric acid and then bromine water in large excess. The precipitate is then collected in tubes by means of a centrifugal machine, washed with bromine water in the same way, dissolved in hot water, and Kjeldahled.

$N \times 6.25 =$ Albumoses and peptones.

Some analysts are in the habit of saturating the filtrate from the coagulable albumenoids with zinc sulphate for the purpose of precipitating the albumoses. In that case the nitrogen so obtained is

¹ The last previous meeting was held March 11. See BIOCHEMICAL BULLETIN, 1915, iv, p. 174.

deducted from the nitrogen contained in the bromine precipitate and the residue is calculated as peptone.

It will be interesting to have the views of the Meeting as to whether this particular separation has any practical utility.

When gelatine is present this is also precipitated by the bromine and the amount as determined below will obviously have to be subtracted from the total precipitate in order to arrive at the percentage of albumoses and peptones.

Gelatine. Five to twenty grammes are dissolved in hot water and evaporated to dryness with sand. The dried mass is then ground finely in a mortar, placed in a beaker, and washed four times with ice-cold alcohol, about 50 c.c. of alcohol being used for each washing. The alcohol is pumped through an asbestos filter surrounded with ice. The sand is then extracted several times with ice-cold alcohol and water, gradually decreasing the strength of the alcohol with each extraction until the final washing-solution contains only 10 percent of alcohol by weight. In all about 5-6 washings of about 50 c.c. each are required. The gelatine in the beaker and on the asbestos filter is then dissolved in boiling water and the nitrogen determined in the usual manner.

$N \times 5.44 = \text{Gelatine.}$

Creatine and Creatinine. A 10 percent solution of the extract in distilled water is first prepared. Several 10 c.c. quantities of this solution, representing 1 gramme of extract, are pipetted into small beakers and to each 10 c.c. of normal hydrochloric acid are added; the beakers are placed in an autoclave and heated for half an hour at a temperature of 120° C.; the whole of the creatine present is thus converted into creatinine. The conversion may also be carried out by dissolving 10 grammes of the extract in a 100 c.c. flask in about 90 c.c. of one-third normal hydrochloric acid, heating in a boiling water bath for 4 hours, allowing to cool and making up to 100 c.c. Ten c.c. of this converted solution, representing 1 gramme of extract, are used for each colour experiment.

Fifty milligrammes of pure crystallised creatine (which contains one molecule of water of crystallization), are similarly converted with hydrochloric acid and the solution made up to 100 c.c. To the contents of one of the beakers, or to the solution of one gramme of

extract otherwise converted, cooled to 20° C., 30 c.c. of a saturated picric acid solution and 15 c.c. of a 10 percent sodium hydroxid solution are added. After standing for 5 minutes the coloured liquid is made up to 500 c.c. The colour is matched in any suitable colourimeter against that given by the standard creatinine solution. It can also be matched against 8 millimetres of a solution of potassium bichromate containing 24.54 grammes of bichromate per litre, the colour of which corresponds with 10 milligrammes of creatine in 500 c.c. of liquid.

Residual Nitrogen usually returned as "Meat Bases." The sum of the percentages of nitrogen existing in the form of coagulable proteins, and albumoses and peptones (and of course gelatine, when present) is subtracted from the total nitrogen: the residual nitrogen multiplied by 6.25 is usually returned as "meat bases." It is clear that when creatine and creatinine are estimated, the nitrogen present in these substances may be deducted from the total nitrogen, in which case the expression "meat bases" must be qualified by the additional words "other than creatine and creatinine." Perhaps, however, the best mode of expression is to state the percentage of "meat bases" as above, and to point out in an independent statement that this contains such and such a percentage of combined creatine and creatinine expressed as creatinine. It is suggested that no useful purpose is to be served by making separate estimations of these two bases.

It will be interesting to have the views of the Members on the employment of the arbitrary factor 6.25 for the conversion of residual nitrogen into the equivalent of so-called "meat bases" and also on the question of the food value of the various groups of constituents mentioned in the above classification.

In order to make the discussion as practical as possible, it is particularly requested that proposals in reference to new or alternative processes of analysis should not be introduced except on the basis of experimental data.

II. PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS, AND THE BIOCHEMICAL SOCIETY, IN JOINT SESSION

Reported by E. Richards Bolton, Joint Hon. Secretary

May 5. SOCIETY OF PUBLIC ANALYSTS and OTHER ANALYTI-

CAL CHEMISTS. 1. Ordinary meeting: held at the Chemical Society's rooms, Burlington House, London. Mr. A. Chaston Chapman, President, in the chair.

The following were elected members of the Society:

Honorary Members—Sir William Crookes and Prof. Meldola.

Ordinary Members—Messrs. Paul Seidelin Arup, Francis Howard Carr, Alexander Scott Dodd and Harri Heap.

Certificates were read for the first time in favour of Prof. Arthur William Crossley, Mr. Daniel James Davies, Dr. Martin Onslow Forster, Prof. Herbert Jackson, Mr. Frederic Ion Richardson, Prof. William Jackson Pope, Prof. James Charles Philip and Mr. George Henry Warburton.

2. The ordinary meeting was followed by a joint meeting of THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS, AND THE BIOCHEMICAL SOCIETY. Mr. A. Chaston Chapman, representing both Societies, in the Chair.

The Meeting was devoted to a discussion on "*Methods adopted in the estimation of the nitrogenous constituents of extracts derived from albuminous substances, such as meat extracts and similar products, with special reference to the interpretation of the results.*"

The discussion was opened by the *Chairman (Mr. Chapman)*, who drew attention to the necessity of dividing the products of protein hydrolysis into certain groups or categories for analytical purposes. From the technical point of view the purposes to be served by such analyses were, firstly, to indicate the general character of the process by which any particular extract had been prepared; secondly, to throw some light on the source of the extract and its genuineness or otherwise; and lastly to furnish information as to the physiological properties or dietetic value. The bromine method, properly applied, precipitated gelatine, gelatine-peptone, syntonin, albumoses and peptones, but not creatinine or other 'meat-bases.' Saturation of the solution with zinc sulphate in presence of a little acid might be resorted to for the purpose of separating the albumoses from the peptones; and this process, although its results were not very definite, might be useful in so far as it threw some light on the extent to which the original protein matter had been hydrolysed. Bigelow and Cook, in the United States, precipitated the albumoses and peptones by

means of tannin in the presence of salt; and, the albumoses being precipitated by zinc sulphate, the peptones were obtained by difference. For the determination of gelatine, the ice-water-alcohol method of Stutzer was perhaps the most used and most generally considered to give the best results, though some inaccuracy might be caused by the fact that substances rich in albumoses yielded an appreciable proportion of their nitrogen by this method.

As to the determination of creatine and creatinine there seemed little to be said from the chemical side, Folin's method having been thoroughly worked out. The use of ammonium salts had been occasionally alleged, but no case of this very crude form of adulteration had come under his notice, though he had met with a sample of extract which, on distillation with magnesium oxide, yielded a considerable quantity of ammonia, due, apparently, to some putrefactive change. The residual nitrogen was often returned as 'meat-bases,' some analysts using for conversion the factor 3.12 originally suggested by Stutzer on the basis of the nitrogen percentage of creatine. Hehner, however, had suggested the use of the ordinary protein factor of 6.25, which at least had the merit of not involving any assumption or giving to the results an apparent accuracy they did not in fact possess; though on the other hand, to those who did not understand the matter, the quantity of 'meat-bases' might be made to appear much larger than it really was, while a considerable error was thrown on the proportion of non-nitrogenous extractives, which was arrived at by difference. The best plan was to return the actual nitrogen percentages.

Prof. F. Gowland Hopkins said that the animal body dealt, not with the intact proteins, or even with the albumoses and peptones, but with the free amino-acids, which were the individual constituents of the protein molecule. The actual way in which the different amino-acids were grouped in the protein molecule was not of much consequence, but the effects produced by the individual amino-acids were of extreme importance. He described physiological experiments which he had made showing that when rats were given a diet including a complete amino-acid mixture corresponding to the proteins of an ordinary diet, their growth was almost exactly normal, while, when arginine and histidine were removed from the amino-acid mix-

ture, the growth ceased immediately, being resumed when arginine and histidine were again added. The removal of tryptophane from the amino-acid mixture also produced similar results; and Osborne and Mendel in America had shown cystine to be similarly essential. It did not follow that this was the case with every amino-acid, and the question as to which of the amino-acids were essential in that way offered a large field for investigation. Recent work at Cambridge indicated that certain acids could be removed from the amino-acid mixture without affecting the rate of growth. With regard to the minimum quantity of any amino-acid required for nutrition, experiment had shown that, in the case of rats, at any rate, the critical minimum for arginine lay somewhere between 2.5 and 1 percent. It appeared that the functions of the individual amino-acids were not confined merely to flesh formation. The effect of feeding animals on zein, which was deficient in both tryptophane and lysine, was not only to restrict growth but also to shorten the survival, and the same was observed with zein *plus* lysine; but with zein *plus* tryptophane the animal was able to maintain its weight for a long period, although it did not grow. It was clear from this that the tryptophane exercised some other function than the mere supply of material for growth. Further work on this subject is proceeding at Cambridge.

Dr. E. P. Cathcart, referring to creatine and creatinine, said that the observations at present available were so scanty that it could not be stated with certainty that creatine and creatinine had a special niche in the organism. Creatinine, of course, was a constant excretory product, but creatine was not, except in the case of infants, though it might be made to appear if an animal were starved or treated with certain drugs. Folin had stated that creatine was a fairly valuable food-stuff, but he (*Dr. Cathcart*) did not think, on the evidence available, that it was. He did not think that any end would be gained by the separate estimation of creatine and creatinine in meat extract, since a large part of the original creatine would probably be converted into creatinine during the process of manufacture.

Mr. A. R. Tankard thought that it was important in some cases to separately estimate the meat fibre and coagulable albuminoids, with a view to the detection of extraneous matters which were some-

times added. For some time past he had adopted the method of centrifugalising the bromine precipitate, mixing the residue with water, and centrifugalising again. He made a practice of separating the albumoses and peptones, but agreed that in most ordinary cases this was of little value. There were, however, on the market certain so-called 'fortified' meat extracts which contained albumoses and peptones in very much larger proportion than occurred in ordinary meat extracts. With regard to the factor used for converting the residual nitrogen into 'meat-bases' he remarked that this was the only instance which he knew of in chemistry in which a factor known to be wrong was used merely for convenience.

Mr. E. Hinks remarked that the use of a factor of approximately 3 for calculating the 'meat-bases' from the residual nitrogen seemed to be justified by the fact that the proportions of nitrogenous and non-nitrogenous extractives thus obtained were about equal, this being what one would expect in the case of such a product as meat extract.

Dr. Percival Hartley described his experience of Van Slyke's method of determining amino-nitrogen, which was based on the fact that when protein and protein degradation products were treated with nitrous acid, nitrogen gas was given off. The results of complete analyses of various proteins showed that the constitution of serum albumen was quite different to that of globulins, the former containing a much larger proportion of lysine and of cystine than the latter. Another interesting result was that the globulins exhibited no difference in chemical composition—euglobulin and pseudoglobulin apparently being closely related. Another point brought out was that the free amino-nitrogen of native proteins appeared to be approximately one-half of the lysine content, which if substantiated, would appear to afford a simple method of estimating lysine without hydrolysis of the protein. Furthermore, the proportion of free amino-nitrogen indicated to what extent the protein had been digested or peptonised.

Further remarks were made by *Dr. Rideal, Prof. Barger, Prof. Harden, Dr. G. S. Walpole, Dr. Cathcart, Prof. Gowland Hopkins and the Chairman.*

III. SCIENTIFIC PROGRAM OF THE BIOCHEMICAL SOCIETY,
JUNE 12

June 12. ROTHAMSTED EXPERIMENT STATION, HARPENDEN,
HERTS.

W. E. Brenchley: The effect of the concentration of nutrient solutions upon the growth of plants in water culture.

W. A. Davis: The periodic variation of the sugars in the foliage leaves of plants during the day and night.

W. A. Davis and *G. C. Sawyer*: The variation of the starch content of the potato leaf during day and night and its relation to the sugars present.

E. H. Richards: The loss of nitrogen during the bacterial decomposition of the nitrogen compounds of animal excretions.

A. Appleyard: The $\frac{\text{CO}_2}{\text{O}_2}$ ratio in soil oxidations.

W. Weir: The effect of soluble humus on plant growth.

E. Horton: Methods for the extraction of organic compounds from soil.

J. Prescott and *E. J. Russell*: The reaction between soil phosphates and dilute acids.

IV. PROVISIONAL SCHEDULE OF MEETINGS FOR 1915-'16

May 5. Joint Meeting with Society of Public Analysts, Chemical Society, Burlington House, London, W.

June 12. Rothamsted Experimental Station, Harpenden, Herts.

Nov. 10. Physiological Laboratory, King's College, London.

Dec. 14. Lister Institute, London.

Feb. 12. University of Leeds.

March 9. Institute of Physiology, University College, London.

V. OFFICERS, 1915-'16

Committee:² *Hon. Treasurer*, J. A. Gardner; *Hon. Secretary*, R. H. A. Plimmer; *Editors of the Biochemical Journal*, W. M. Bayliss and A. Harden; *Ordinary Members*, G. Barger, V. H.

² The business of the Society is conducted by a committee consisting of a treasurer, secretary, the editors of the *Biochemical Journal*, and twelve ordinary members.

Blackman, A. Chaston Chapman, W. A. Davis, A. E. Garrod, W. D. Halliburton, T. A. Henry, W. H. Hurtley, G. W. Monier-Williams, J. Lorrain Smith, H. M. Vernon, T. B. Wood.

VI. LIST OF THE MEMBERS ELECTED SINCE THE PUBLICATION,
IN THE BIOCHEMICAL BULLETIN, OF THE ORIGINAL
LIST OF MEMBERS³

- ANREP, DR. G. VON, Univ. College, London, W. C.
 ATKIN, DR. E. E., Lister Institute, Chelsea Gardens, London, S. W.
 AULD, PROF. S. M. J., University College, Reading.
 BOTTOMLEY, PROF. W. B., King's College, Strand, London, W.C.
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 London, S.W.
 DUDLEY, DR. H. W., The University, Leeds.
 EDIE, E. S., Esq., B.Sc., The University, Aberdeen.
 EULER, PROF. H. VON, Stockholms Högskola, Stockholm, Sweden.
 FINLOW, R. S., Esq., B.Sc., Dacca, Bengal, India.
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 HINKS, E., Esq., F.I.C., 16 Southwark Street, London, S.E.

³ BIOCHEMICAL BULLETIN, 1913, ii, p. 447.

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- MACALLUM, DR. A. BRUCE, University, Toronto, Canada.
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University College, London.

AMERICAN CHEMICAL SOCIETY.

Proceedings of the Division of Biological Chemistry, New Orleans, La., March 31–April 3, 1915.¹

REPORTED BY

PAUL E. HOWE

The American Chemical Society held its fiftieth meeting in New Orleans, La., March 31–April 3, 1915. The Division of Biological Chemistry held its meeting at the Hotel Grunewald, on April 2nd. The following papers were presented:

C. L. Alsberg: Control of cotton-seed products.—*C. C. Bass*: Emetine, the specific remedy for *pyorrhœa alveolaris*.—*Charles Mann*: Saw palmetto, a biochemical study.—*E. R. Miller and J. M. Moseley*: Volatile oils of some species of *Solidago*.—*E. R. Miller*: Volatile oils of several species of *Eupatorium*; Volatile oil of *Achillea millefolium* L; Some volatile oils from the genus *Pycnanthemum*.—*W. J. V. Osterhout*: Artificial photosynthesis by chlorophyl.—*W. E. Tottingham*: Rôle of chlorin in plant nutrition.—*G. S. Fraps*: Nitrification studies.—*C. B. Lipman*: The form of nitrogen in nitrogenous materials as an index to nitrifiability.—*M. X. Sullivan*: Formation of creatinin by bacteria; Amount of creatinin in plants.—*Edward Gudeman*: Toilet papers, a source of infection.—*Adolph Bernard*: Simple colorimetric method for the determination of free reducing sugars and total carbohydrate in miscellaneous food products.—*J. P. Atkinson*: Reducing action of certain carbohydrates on distillation.—*Sara S. Graves*: Precipitant for ammonia.—*W. Denis*: Phenols and phenol derivatives in urine.—*J. H. Long*: Physiological activity of combined hydrochloric acid; Combinations of proteins with halogen acids.—*Charles Baskerville*: Rate of evaporation of ether from oils and its application in oil-ether colonic anesthesia.—*H. S. Grindley and E. C. Eckstein*: Free amid nitrogen and

¹ For accounts of the organization of the Biochemical Division and successive meetings see *BIOCHEM. BULL.*, 1911, i, p. 94; 1913, iii, p. 76; 1914, iii, p. 444.

the free amino-acid nitrogen of feedingstuffs.—*H. S. Grindley, W. E. Joseph and M. E. Slater*: Quantitative determination of the amino-acids of the mixed proteins of feedingstuffs.—*R. S. Potter and R. S. Snyder*: Nitrogen distribution according to the Van Slyke method in soils and their "humic acids"; Amino-acid nitrogen in soils variously treated.—*Max Kahn*: Urinary mucin.—*M. Kahn and F. G. Goodridge*: Cystin.—*F. G. Goodridge*: Biochemical studies of mercaptan.—*M. Kahn and Francis Huber*: Metabolism studies of multiple myeloma with Bence-Jones albumose.—*M. Kahn and S. Schneider*: Study of the mineral metabolism of diabetics.—*A. F. Hess and M. Kahn*: Mineral metabolism of two cases of hemophilia.—*Jacob Rosenbloom*: Ethereal sulfates of the urine in various diseases; Modification of Gerhardt's test for diacetic acid; Influence of low and high protein intake on the excretion of acetone, diacetic acid, and β -hydroxybutyric acid in diabetes.—*W. M. Clark*: Adjustment of the reaction of bacteria culture media; Final hydrogen-ion concentration of cultures of *B. coli*.—*E. H. Walters and W. M. Clark*: Relation of propionic fermentation to the development of "eyes" in Ementhaler cheese.—*S. L. Jodidi*: Factor to be used for the calculation of the phosphoric acid in Neumann's method.—*S. L. Jodidi and E. H. Kellogg*: Factor to be used for the calculation of phosphoric acid in Neumann's method. I. Factor as influenced by the water used for washing the yellow precipitate.—*R. E. Swain and E. R. Harding*: Quantitative estimation of allantoin.—*Lewis Knudson*: Influence of certain sugars on the growth and respiration of vetch.—*W. J. Robbins*: Influence of certain inorganic substances on the digestion of starch by *Penicillium camemberti*.—*W. M. Clark and H. A. Lubs*: Differentiation of organisms of the colon group by means of indicators.—*C. C. Johns and Arno Viehoveer*: Studies on the saponins of *Chlorogalum pomeridianum* and of *Agave lechuquilla*; Alkaloids of *Amianthium muscatorium*.

Biochemical Laboratory of Columbia University,
College of Physicians and Surgeons, New York.

AMERICAN PHILOSOPHICAL SOCIETY

General meeting—April 22 to 24, 1915

REPORTED BY

JOSEPH S. HEPBURN

NEW MEMBERS.—The following, among others, were elected members: *John J. Abel* (Johns Hopkins Univ.), *John M. Coulter* (Univ. of Chicago), *William J. Gies* (Columbia Univ.), *Philip B. Hawk* (Jefferson Med. Coll., Phila.), *Thomas H. Morgan* (Columbia Univ.), *Raymond Pearl* (Maine Agric. Exp. Station), *Theobald Smith* (Harvard Univ.).

PAPERS OF BIOLOGICAL AND CHEMICAL INTEREST.—*M. H. Jacobs*: Heredity in protozoa.—*T. H. Morgan*: Constitution of hereditary material.—*G. H. Parker*: Problem of adaptation as illustrated by the fur seals of the Pribilof Islands.—*Edward M. East*: Interpretation of sterility in hybrids.—*G. H. Shull*: Heterosis and the effects of inbreeding.—*B. M. Davis*: Significance of sterility in *Oenothera*.—*G. F. Atkinson*: Morphology and development of *Agaricus rodmani*.—*William Trelease*: Large-fruited American oaks.—*M. V. Cobb*: Relationships of the white oaks of Eastern North America.—*L. H. Bailey*: Present need in systematic botany.—*M. T. Bogert*: Convenient form of receiver for fractional distillations under diminished pressure.—*J. R. Tuttle* and *M. T. Bogert*: Cymene carboxylic acids.—*E. Plaut* and *M. T. Bogert*: Syringic acid and its derivatives.—*W. J. Gies*: Relation of ductless glands to dentition and ossification.—*P. B. Hawk*: Gastro-intestinal studies.—*Charles Baskerville*: Rate of evaporation of ether from oils and its application in oil-ether colonic anesthesia.—*A. J. Smith*: Oral endamebiosis.—*J. T. W. Marshall*: New form of nephelometer.—*W. M. Davis*: New evidence for Darwin's theory of coral reefs.—*Stewart Paton*: Certain factors conditioning nervous responses.

Philadelphia, Pa.

BIOCHEMICAL NEWS, NOTES AND COMMENT

EDITORIAL SUB-COMMITTEE:

Benjamin Horowitz,

William J. Gies, Hattie L. Heft, Joseph S. Hepburn,

Paul E. Howe, Edgar G. Miller, Jr., William A. Perlzweig

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(II). *War Notes*: Necrology, 443; university items, 444; booze bombs, 444; honors, 445; notes on the sanitation of military quarters and battlefields, 446; food notes, 447; medical notes, 450; miscellaneous items, 453.

(III). *Col. Univ. Biochem. Assoc.*: (1) General notes—honors, 453; appointments, 454; dental graduates, 454; associations and societies, 454; miscellaneous items, 455; (2) Proc. of the Assoc.—23^d (sixth annual) meeting, 456; (3) Columbia Biochem. Dep't—appointments, 456; associations and societies, 457; addresses and reports, 457; awards of higher degrees, 458; summer session, 459; miscellaneous items, 459.

I. GENERAL

Necrology.¹—*A. Arnaud*, who occupied the chair of chem. at the Museum of Nat. Hist., Paris; distinguished for work in chem. and pharmacol.

Paul Ehrlich, director, Royal Inst. for Exp. Therapeutics, Frankfort.

Erich Harnack, director, Inst. of Pharmacol., Halle.

A. Sheridan Lea, formerly Univ. lecturer in physiol., Cambridge; well known for his researches in physiol. chem., and his "Chemical basis of the animal body."

Friedrich Löffler, director, Robert Koch Inst. for Infec. Diseases, Berlin; discoverer of the diphtheria bacillus.

John U. Nef, head of the dep't of chem., Univ. of Chicago; eminent for his contributions to organic chem.

Albert Plaut, vice-p., N. Y. Coll. of Pharmacy.

Dr. Proskauer, director, Berlin Bureau of Food and Trades

¹ War necrology, page 443.

Inspection; an authority in the field of water-supplies and sewage-disposal.

Ludwig Tobler, chief, Children's Hosp., Breslau; investigator of the pathol. of nutrition.

BODY OF PROF. HILDEBRANDT RECOVERED. In 1912, Dr. *Hildebrandt*, instructor in pharmacol., Univ. of Halle, lost his life during a mountain-climbing trip. His body was recently found in a crevice, by soldiers crossing the Bozen district.

Appointments.² Digestive Ferments Co. (Detroit, Mich.): *J. W. M. Bunker* (Harvard Univ.), direc., bacteriol. research dep't.

Fordham Univ., Med. Sch. (N. Y. City): *Lewis W. Fetzer* (U. S. Dep't of Agric.), prof., physiol. and biochem., vice *Alwyn Knauer*, resigned; *Carl P. Sherwin*, assis. prof., biochem.; *John A. Killian*, instr., physiol. and biochem.

McGill Univ., Med. Sch.: *F. W. Skirrow*, assis. prof., chem.: *J. C. Meakins* and *F. B. Gurd*, lect's, immunology.

Mass. Agric. Coll.: *Paul Serex*, instr., chem.

Montana Agric. Coll.: *R. H. Bogue* (Mass. Agric. Coll.), assis. prof., agric. chem.

N. Y. City Dep't of Health: *L. P. Brown* (State Food and Drug Commiss., Tenn.), direc., Bur. of Food Inspec.; *Matthias Nicoll, Jr.*, assis. direc. of lab's.

N. Y. Post-Grad. Med. Sch. and Hosp.: *Morris Fine*, adj. prof., path. chem. (prom.).

Okla. Agric. and Mech. Coll.: *L. C. Raiford* (Univ., Chicago), prof., chem.

Parke, Davis and Co. (Detroit): *S. P. Miller* (Mass. Agric. Coll.), chemist.

Queen's Univ., Med. Sch. (Kingston, Ont.): *J. O. Halverson* (Jeff. Med. Coll.), lect., biol. chem.

Rockefeller Inst. Med. Research. ASSOCIATES (promotions): *C. J. West*, chem.; *Michael Heidelberger*, chem.; *Angelia M. Courtney*, chem.—ASSISTANTS (promotions): *C. H. Allen*, chem.; *J. K. Senior*, chem.; *G. E. Cullen*, chem.; *Marian Vinograd*, chem.—NEW APPOINT'S: *E. A. Wildman*, fell., chem.; *A. L. Meyer*, assis., physiol. and pharmacol.

²In this summary institutions from which appointments were made are named in parenthesis. See also pages 454 and 456.

Sta'n for Exp. Evol., Carnegie Inst'n: *A. F. Blakeslee* (Conn. Agric. Coll.), plant geneticist.

U. S. Dep't of Agric., Bur. of Chem.: *F. B. La Forge* (Rockefeller Inst.), expert, org. chem. Food Research Lab. (Phila.); *T. E. Harper, Jr.*, chemist's aid, fish-handling investigations.

U. S. Pub. Health Serv., Pellagra Hosp. (Spartanburg, S. C.): *J. R. Murlin*, temp. biochem.; *M. X. Sullivan* (Div. of Soil Fertility, U. S. Dep't of Agric.), permanent biochem.

Univ., Cal.: *H. T. Chickering* (Rockefeller Inst.), "research associate in pathol." He will be associated with Prof. F. P. Gay in investigations on the treatment of typhoid by the use of sensitized vaccine. This research associateship, established with a gift by Mr. J. K. Moffit, one of the Univ's regents, is additional to a research associateship in pathol., for which other donors recently agreed to provide an annual gift of \$1,200, and an eventual endowment of \$25,000. Hooper Foundation for Med. Research: *S. H. Hurwitz* (Harvard Med. Sch.): instr., research med.

Univ., Halle, Pharmacol. Inst.: *R. Magnus* (Utrecht), prof., pharmacol. and direc., in succession to Prof. *Harnack*, deceased.

Univ., Ill.: *H. B. Lewis* (Univ. of Penn.), assoc., physiol. chem. Agric. Exp. Sta.: *Robert Stewart* (Utah Agric. Coll.), assoc. prof., soil fertility; assis. chief, soil fertility work.

Univ., Minn.: *Jean MacKinnon*, assis. prof., nutrition.

Univ., Oregon: *H. B. Myers* (N. Y. Univ. and Bell. Hosp. Med. Coll.), prof., mat. med. and pharmacol.

Univ., Penn.: *A. I. Ringer*, assis. prof., physiol. chem. (*resigned*); *R. L. Stehle* and *B. M. Hendrix*, instrs., physiol. chem.

Univ., So. Cal., Sch. of Med.: *C. G. MacArthur*, instr., biochem. and toxicol.

Univ., W. Va., Coll. of Agric., Exp. Sta'n: *J. L. Coulter* (Geo. Peabody Coll.), dean, Coll. of Agric.; direc. Exp. Sta'n, vice *E. D. Sanderson*, resigned.

Va. State Dep't of Agric.: *J. B. Weems*, chief chemist, to succeed Dr. *Magruder*, resigned.

West. Reserve Univ., Med. Sch.: *P. J. Hanslik*, assoc., pharmacol. (prom.); *G. E. Simpson*, instr., org. and biol. chem. (prom.); *H. H. McGregor*, instr., biochem.; *R. J. Collins*, demonstr., pharmacol.

Yale Univ., Med. Sch. : *C.-E. A. Winslow*, Anna M. L. Lauder prof. of public health. He will resign his positions in the N. Y. State Dep't of Health and Teachers' Coll., to take up this work in the fall, but will continue to act as curator of public health at the Amer. Mus. of Nat. Hist., N. Y. City.

Honors. POSTHUMOUS. A monument to the late Prof. *J. H. van't Hoff* was unveiled at Rotterdam, Apr. 17. It consists of a bronze statue, double life-size, in sitting position, placed in front of the school at which van't Hoff was educated. The monument is about 30 ft. high, and the statue itself is flanked by female figures representing Imagination and Reason. On the front of the base is the following inscription:

Van't Hoff
1852-1911
Physicium chemiae adiunxit

The centenary of the birth of *David Waldie*, who suggested, to Sir James Simpson, a trial of chloroform as an anesthetic, has been commemorated with a bronze tablet on the house in Linlithgow where he lived for some time. The tablet shows a portrait of Waldie, with an inscription in which he is described as a pioneer in anesthetic research.

HONORARY DEGREES. Washington Univ. (Med. Sch.), Apr. 30: ScD., *Otto Folin*; LL.D., *R. H. Chittenden*, *S. J. Meltzer*, *W. H. Howell*.

V. C. Vaughan, LL.D., Jeff. Med. Coll.

John A. Kolmer, M.S., Villanova Coll.

F. E. Stewart, Phar.D., Medico-Chi. Coll., Phila. Dr. Stewart is prof. of mat. med. in the Medico-Chi. Coll. and organizer of the Nat'l Bur. of Med. and Foods.

Phila. Coll. of Pharm., Pharm. M. : *E. G. Eberle*, ed., *Jour. Amer. Pharmaceut. Assoc.*; *C. A. Mayo*, pres., Amer. Pharmaceut. Assoc., ed. of *Amer. Druggist*; *W. M. Mittlebach*, pres., Nat'l Assoc. Boards of Pharmacy; *H. M. Whelpley*, treas., Amer. Pharmaceut. Assoc. and Board of Trustees of U. S. Pharmacop. Conven., prof. of microscopy and dean of St. Louis Coll. of Pharm., prof. of mat. med. and pharm. of Washington Univ.; *W. L. Cliffe*, trustee, Phila. Coll. of Pharm.

DINNERS AND CELEBRATIONS. A testimonial dinner was tendered to Dr. *Leo Loeb* at the Univ. Club, St. Louis, May 25, by members of the med. profession of St. Louis, the scientific faculties of Washington and St. Louis Univ's, and members of the Biol. Society of St. Louis.

Dr. *J. P. Lobenhoffer*, on June 15, completed 15 years of continuous service as chemist at the Touro Infirmary, New Orleans. He was presented by his assistants with a gold smoking set and the Board of Direc. adopted a resolution expressing their keen appreciation of his services.

Prof. *J. M. Bartlett*, on April 30, completed 30 years of continuous service as chemist at the Maine Agric. Exp. Sta'n. This period includes the entire history of the station. In recognition of this unusual length of service in one institution, a reception in Prof. Bartlett's honor was held in the station building on the evening of Apr. 30, and he was presented with a commemorative volume. This volume was composed of a series of congratulatory letters from nearly all of the 109 different persons, now living, who have, at one time or another, been associated with Prof. Bartlett in the work of the station.

A celebration which, owing to present circumstances, has taken on a personal character, was recently held at the Pasteur Inst.; the occasion was the 70th birthday anniv. of Prof. *Elie Metchnikoff*. Prof. Gaston Darboux and Dr. Roux, on behalf of the Acad. des sciences and Pasteur Inst., resp., reviewed the career and the works of the Russian scientist. Prof. Metchnikoff gave the audience an interesting talk on the prolongation of life. PARIS LETTER: *Jour. Amer. Med. Assoc.*, 1915, lxiv, p. 2082.

MISCELLANEOUS. The name of *Curie*, in honor of the discoverer of radium, has been given to a small park (Place de Curie) formed by the demolition of the old rue Dauphine in Paris.—Royal Roumanian crosses of the first class for *Sanitätsverdienst* (merit in sanitary service) have been conferred on *von Behring* and *Ehrlich*.

Lectures and addresses. CUTTER LECTURES. The Cutter Lectures in preventive medicine, for 1915, were given at the Harvard Med. Sch. by *V. C. Vaughan* and *Joseph Goldberger*. Dr. Vaughan discussed The phenomena of infection, April 14, 15 and 16; Dr. Goldberger, Diet and pellagra, in one lecture, April 12.

MISCELLANEOUS ITEMS. Cleveland Acad. of Med., May 21: *R. M. Pearce* (Univ. of Penn.), Relation of the spleen to blood destruction and regeneration, and to hemolytic jaundice.

Coll. of Phys. and Surg. (Phila.), April 12: *V. C. Vaughan* (Univ., Mich.), Phases of modern military hygiene and camp sanitation, particularly in reference to war mortality.

Columbia Univ.; *Phi Lambda Upsilon Lect.*, May 6: *David T. Day* (U. S. Bur. of Mines), Petroleum, illustrated with motion pictures.

Jeff. Med. Coll., June 15: *V. C. Vaughan* (Univ., Mich.), A doctor's ideals.

King's Coll. (London), May 31, June 2, 7 and 9: *T. B. Brodie* (Univ., Toronto), Gases of the blood.

N. Y. Bot. Gard. Summer series: *Wm. Mansfield* (N. Y. Coll. of Pharm.), Poisonous plants of eastern U. S. Late-summer series: *W. A. Murrill*, The use of mushrooms for food.

Ohio State Univ., Apr. 9: *A. J. Carlson* (Univ., Chicago), Recent contributions to the physiology of the stomach.

Univ., Chicago; *Phi Lambda Upsilon Lect.*, May 21: *J. U. Nef*, Chemistry of enzyme action.

Vassar Coll., May 10: *F. G. Benedict* (Carnegie Nutr. Lab.), (1) Investigations in the Nutr. Lab. of the Carnegie Inst'n of Washington; (2) Women as research assistants.

Washington Univ., Med. Sch. (dedication week, April): *Otto Folin* (Harvard Med. Sch.), (1) The utilization of food protein; (2) Tissue metabolism, with special reference to creatinin; (3) Protein metabolism, with special reference to uric acid; (4) The occurrence and significance of phenols and phenol derivatives in the urine.

Medals. BALY MEDAL, Royal Coll. of Phys. (London): To *F. G. Hopkins*.—DAVY MEDAL (Royal Soc.): To *W. J. Pope*, for his researches on stereochem. and on the relations between crystal-line form and chem. structure.—FRANKLIN MEDAL (the highest recognition in the gift of the Franklin Inst., Pa.): To *H. K. Onnes*, for his low-temp. research, and to *T. A. Edison*, for his "numerous basic inventions and discoveries forming the foundation of world-wide industries, signally contributing to the well-being, comfort and pleasure of the human race."

The bronze thesis-medal of the SCIENCE CLUB, UNIV. OF WIS., was awarded, at commencement, to *Walter Pitz* for a thesis on The effect of elemental sulfur and of calcium sulfate on certain of the higher and lower forms of plant life. This medal is awarded annually to a senior in the Univ. of Wis. for quality and quantity of research in the preparation of a thesis in physical or natural science, or pure mathematics, or their useful applications.

Prizes. The Royal Soc., Edinburgh, has awarded the **MAK-DOUGALL-BRISBANE PRIZE**, for 1912-14, to *C. R. Marshall* (Dundee and St. Andrews), for his studies on the pharmacol. action of tetra-alkyl ammonium compounds.

The **OSIRIS PRIZE**, \$20,000, which the Inst. of France gives every three years as a reward for the most remarkable work or discovery by Frenchmen in science, art, letters or industry, was awarded jointly to Prof's *Widal* ($\frac{1}{4}$) and *Chantemesse* ($\frac{1}{4}$), and Dr. *Vincent* ($\frac{1}{2}$), whose names are connected with the development of anti-typhoid inoculation. As the Osiris prize can be given only to Frenchmen, the Inst. decided to award, in this connection, a special prize to Sir Almroth Wright, who first prepared anti-typhoid serum.

Associations, societies, etc.: Officers-elect. AMER. ASSOC. IMMUNOLOGISTS. Pres., *J. W. Jobling*; vice-p., *G. P. Sanborn*, *W. T. Councilman*, *J. A. Kolmer*; treas., *W. J. Stone*; sec., *M. J. Symott*.

AMER. MED. ASSOC. Pres., Surg. Gen., *R. P. Blue*.

AMER. NEUROLOG. ASSOC. Pres., *L. F. Barker*.

AMER. PHARMACEUT. ASSOC. Pres., *F. L. Wulling*, dean, Coll. of Pharm., Univ. Minn.

AMER. SOC. TROP. MED. Pres., *M. J. Rosenau*.

ASSOC. AMER. PHYSICIANS. Pres., *Henry Sewall*.

GERMAN ASSOC. SCIENTIFIC MEN AND PHYSICIANS. Pres., *F. von Müller*.

MEDICAL BROTHERHOOD. See page 294.

NEW ORLEANS ACAD. SCIENCES. Pres., *Gustav Mann*.

SIGMA XI, DIST. COLUMBIA CHAPT. Vice-p., *Isaac K. Phelps*.

SOC. EXP. BIOL. AND MED. Pres., *Graham Lusk* (re-elec.); vice-p., *G. N. Calkins*; sec-treas., *H. C. Jackson*; addit. members of the Council, *Wm. J. Gies* and *John Auer*.

Members elect. AMER. PHILOSOPH. Soc.: J. J. Abel, J. M. Coulter, Wm. J. Gies, P. B. Hawk, Theobald Smith.

NAT'L ACAD SCIENCES. F. R. Lillie, Graham Lusk, Alexander Smith, V. C. Vaughan.

SOC. EXP. BIOL. AND MED. G. M. Baehr, Olaf Bergeim, Warren Coleman, D. J. Edwards, P. A. Kober, J. A. Kolmer, H. B. Lewis, W. G. Lyle, S. S. Maxwell, W. F. Peterson, G. H. Whipple.

Miscellaneous items. PERSONAL. Dr. *Rokuro Nakaseko* (M.S., Yale: Ph.D., Johns Hopkins) has lately been a visitor in this country. He is now in charge of the A. C. James Lab., Muro-machi-Demizu, Kyoto, Japan.

MERITORIOUS EXHIBIT. The commit. on awards for scientific exhibits, at the San Francisco meeting of the Amer. Med. Assoc., granted a gold medal to Dr. M. H. Fischer, for his exhibit on Newer experiments in the physiology and pathology of kidney functions.

LAB. KINKS. *Method of paraffining labels.* Instead of going to the trouble of melting paraffin whenever you wish to apply this as a protective coating, the paraffin can be dissolved in carbon tetrachlorid, making a saturated sol. This may be applied as a varnish. The carbon tetrachlorid evaporates, leaving a coating of paraffin behind. Gasoline or benzine might be used but carbon tetrachlorid is preferred, *as its vapor is not inflammable.* D. L. RANDALL: *Chemist-Analyst*, 1915, no. 13, p. 25.

Cleaning solution for glass and porcelain. The following sol. for removing org. material from glass- or porcelain-ware has been found by the author much superior to the well-known potassium-bichromate-sulfuric-acid mixture. The glass- or porcelain-appar. to be cleaned is placed in a large evap. dish containing sulfuric acid and a little nitric acid. If the appar. is not completely immersed, it is turned after a few minutes so that the cleansing sol. comes in contact with every part. The acid should be kept very warm but not hot enough to evolve sulfur trioxid or to distill the nitric acid. Thick glassware, *e. g.*, suction flasks, should be placed in the cold mixture, which is then heated. The sol. is permanent except that enough nitric acid must be added, from time to time, to keep it white or at least yellow in color rather than black. This mixture has been found especially useful in cleaning dyes, gums and waxes from glassware.

A thin film of dye will be removed almost instantly, but the removal of masses of org. material requires longer treatment. F. C. MATHERS: *Chemist-Analyst*, 1915, no. 13, p. 10.

FOOD NOTES. *Boric acid proscribed.* Constitutionality of the Ill. pure-food law, prohibiting, in effect, sale of food preservatives containing boric acid, was upheld, June 21, by the U. S. Supreme Court. Justice Hughes stated, for the court, that the law must be upheld as valid unless the defendant shows there is no doubt that boric acid is wholesome, which, the court held, he had failed to do.

Diet treatment of pellagra. An arrangement has been entered into between the U. S. Pub.-Health Serv. and the Epworth Orphanage, Columbia, S. C., for the application of the diet treatment of *pellagra* among the children, in the orphanage, afflicted with the disease. The Service will prescribe the diet and furnish the protein portions of it. All necessary facilities will be given the gov't officials in this work.

JOHN BARLEYCORN ON THE RUN. *Physicians favor prohibition.* The Acad. of Med., Edmonton, Alberta, has adopted the following temperance resol.: That the Acad. of Med., City of Edmonton, favors prohibition in the Province of Alberta and endorses the proposed liquor act for the suppression of the liquor traffic in Alberta, Can.

"Fine old whiskey" as dangerous as the cheap raw product. The *Weekly Bulletin* of the N. Y. State Health Dep't quotes from the summary of the Investigation concerning the physiological aspects of the liquor problem, by Dr. J. S. Billings, which was prepared for the Commit. of Fifty, showing that the common idea, that a large degree of the injury to health from the use of alcoholic drinks is caused by injurious substances in the liquor, such as fusel oil and furfurool, which have not been properly removed, is erroneous, as is also the notion that cheap liquors contain larger quantities of such ingredients than others. *The injurious effects of the fusel oil are trifling in comparison with those of the ethyl alcohol.* The general conclusion is that "fine old brandies and whiskies" are nearly as likely to produce ill effects as the cheaper varieties of the present time, if taken in the same quantities; and that the injurious effect is in proportion to the ethyl alcohol contained. *Jour. Amer. Med. Assoc.*, 1915, lxxv, p. 885.

N. Y. Health Dep't temperance crusade. The N. Y. City Dep't of Health recently published the results obtained from a study of the effects of alcohol on human life. Forty-three leading life insurance companies have furnished their *records on about 2,000,000 lives*, for a period of 25 years. The report states that nothing has been more conclusively proved than that the steady free use of alcoholic beverages, or occasional excess, is detrimental to the individual. Among men who admitted that they had taken alcohol occasionally to excess in the past, but whose habits were considered satisfactory when they were insured, the extra-mortality was equivalent to a reduction of more than 4 years in the average expectation of life for these men. The report further states that available statistics justify the statement that total abstainers have a mortality during the working years of life of about one-half that for those who take two glasses of whiskey a day.

TOXICOLOGICAL NOTES. Wood alcohol in toilet preparations. Investigation by inspectors of the N. Y. City Health Dep't last year showed that more than one-third of the toilet preparations sold in the city contained wood alcohol, which is forbidden by the Sanitary Code. Of more than 300 preparations taken from barber shops, manicuring establishments and supply-houses, during the past few months, however, only a small proportion contained this deadly poison. Systematic inspections will be continued.

Retail druggists endorse patent-medicine campaign. At a meeting of the pharmacists of N. Y. City, July 2, under the auspices of the Bronx Co. Pharmaceut. Assoc., resolutions were adopted endorsing the Health Dep't's campaign against fraudulent patent medicines. This change in the attitude of the pharmacists of the city is very gratifying. It may now be expected that N. Y. City pharmacists will do all in their power to uphold the Dep't of Health in its endeavor to stop the local sale of the many fraudulent cure-alls now on the market.

Drug victims fill city prisons. At a recent conference on crime and environment, Dr. Katherine B. Davis, Commiss. of Corrections, N. Y. City, stated that on Mar. 1, 1914, there were 4,647 persons in the correctional inst's of that city, but on Mar. 1, 1915, there were approx. 7,500, *an increase of almost 50 percent.* Dr. Davis attrib-

utes this increase to the drug crusades of the past 15 months, which have increased the number of commitments of users of habit-forming drugs as well as of persons convicted of selling narcotics illegally.

FALL IN THE PRODUCTION-COST OF RADIUM. Sec'y of the Interior Lane authorizes the statement that the production of radium from Colorado carnotite-ores by the Bur. of Mines, in connection with the Nat'l Radium Inst., has passed the exper. stage in its new process and is now on a successful manufacturing basis. He says: The cost of 1 gram of radium bromid during Mar., Apr. and May of the present year was \$36,050. This includes the cost of ore, insurance, repairs, amortization, allowance for plant and equipment, cost of Bur.-of-Mines cooperation, and all expenses incident to the production of high-grade radium bromid. When it is considered that radium (bromid) has been selling for \$120,000 to \$160,000 a gram, it will be seen just what the Bur. of Mines has accomplished along these lines.

BOTANICAL NOTES. *N. Y. Botan. Garden anniv.* The 20th anniv. of the opening of the N. Y. Botan. Garden was celebrated by Amer. botanists at the Garden, Sept. 6-11. At the initial meeting, the delegates and visitors were welcomed, on behalf of the B'd of Managers, by Dr. W. Gilman Thompson, pres., and on behalf of the Scientific Directors, by Dr. H. H. Rusby, chairman.

Relation of botany to medicine. "The most important development of modern biology came when the great principle of the existence of cells was transferred *from botany to zoology* by Theodor Schwann, at the beginning of the 19th century. When Virchow took the further step of applying the cell-doctrine to pathol., he made perhaps the greatest advance in modern medicine. He used to declare in later life that when these two far-reaching developments were made by himself and Schwann, the rising generation of scientific investigators in Germany *were quite as much interested in botanic problems as in microscopic anatomy*. It was this breadth of interest, he declared, that gave them the larger outlook which enabled them to see beyond the bounds of what had been hitherto known, to newer phases of knowledge." *Jour. Amer. Med. Assoc.*, 1915, lxiv, p. 2142.

OBESITY OF THE HAND. *Interesting case of fat-metabolism.* A defect in the back of the hand of a girl of 12 was remedied with a flap taken from her abdomen. It healed in place and answered its purpose perfectly until late in life. After 30 she became obese. Then the patch on her hand increased in size proportional to the increasing thickness of the abdominal wall. *Jour. Amer. Med. Assoc.*, 1915, lxiv, p. 2106.

HOMEOPATHIC PROGRESS. The Hahnemann Med. Coll., San Francisco, conveyed all its property to the Univ. of Cal., and has discontinued separate instruction. Instead, two professors will be maintained in the Univ. of Cal. Med. Sch. in homeopathic mat. med. and in homeopathic therap. Instruction in these subjects will be offered as *elective* courses. Students wishing eventually to become homeopathic practitioners will be given the same instruction, in the Univ. of Cal. Med. Sch., as all the other students will receive.

ANTI-VIVISECTION BILL UNCONSTITUTIONAL. Gov. Johnson has declined to approve the *anti-vivisection* bill passed by the Cal. Legislature at its last session. The commit. on med. instr. of the regents of the Univ. of Cal., the deans of the Cal. and Stanford Med. Sch.'s, the biolog. and agric. investigators, the med. practitioners, and many other citizens, had protested against the measure as an unwarrantable interference with science. In declining to approve the bill the Gov. announced that its provision, that any humane officer should be permitted to invade any scientific lab. without a search warrant, was *an unconstitutional interference with personal liberty and the rights of privacy.*

KEEN FELLOWSHIP. Prof. W. W. Keen has established the Corinna Borden Keen Research Fellowship in Jeff. Med. Coll., the income from which now amounts to \$1,000. The gift provides that the recipient of the fellowship shall spend at least one year wherever he can obtain the best facilities for research in the line of work he shall select, after consultation with the faculty, and that he shall publish at least one paper, embodying the results of his work, as the "Corinna Borden Keen Research Fellow of the Jefferson Medical College." Applications, stating the line of investigation which the candidate desires to follow, should be forwarded to Dr. R. V. Patterson, Sub-Dean, Jeff. Med. Coll., Phila.

BIOCHEM. WORK IN THE BUREAU OF SCIENCE, PHILIPPINE ISLANDS. In response to our request for a statement of the nature of the biochem. work done, or in progress, under the auspices of the Philippine Bur. of Science, we have received the following from Acting-Direc., Dr. J. A. Johnston, dated, Mar. 3, 1915: The Bureau of Science has no regular staff devoted to biochem. work and has carried on no work dealing strictly with the chem. of life processes. A few practical problems have involved studies more or less bordering on the biochemical. Of such might be mentioned the investigation of the enzymes of the nipa palm, the constituents of certain foods, active principles of medicinal plants, the etiology of beriberi, the treatment of leprosy, and the relation of soil moisture and environmental conditions to plant growth. All of these are of incidental interest to biochemistry. Many problems still to be attacked seem to offer attractive opportunities to physiol. and pathol. chemists.

A PHARMACEUTIC EXPER. STATION. "During recent years the number of med. schools which have been provided with facilities for research, through state grants or by private endowments, has rapidly increased. While much is still to be hoped for, the outlook for med. advance is far brighter in this respect than it is in the related branch, pharmacy. Pharm. schools, whether privately owned or controlled by the state, are almost without exception devoted to the routine instruction of students and are, as a rule, doing nothing toward the advance of pharmacy as a science. A notable exception to this backward condition of pharmac. education was the establishment of a pharmac. exper. sta'n by the Wis. Legislature, in 1913. The statute creating the exper. sta'n provides that it further the cultivation and investigation of medic. plants. In the first annual report of the direc. of the sta'n,³ Edward Kremers, a well-known authority and author on plant chemistry, gives an indication of what may be expected from this pioneer work. The sta'n not only is engaged in the exper. cultivation of medic. plants, but also cooperates with the gov't in this field, and offers its help and advice to those who wish to engage in this relatively new Amer. industry. While drug-plant cultiv. is to be a prominent feature of the sta'n's scope, the work planned by the

³ Report of the Direc. of the Pharmac. Exper. Sta'n, Bull. 542, Univ. Wis., Dec., 1914.

director has a wider sphere. The present report shows that plant analysis, a most neglected subject, as well as the so-called synthetics, is to receive attention." *Jour. Amer. Med. Assoc.*, 1915, lxxv, p. 259.

A HALF CENTURY OF ANTISEPTIC SURGERY. "In a review of the scientific features in the development of modern surgery, Lee⁴ has written: 'With the discovery of practicable anesthetics, the battle was only half won. The operation itself had lost much of its horror, but the tragedy of the subsequent days was unchanged. There were the almost inevitable suppuration of the wound, the putrefaction and sloughing off of tissue, the sickening odor, the high fever, the danger of hemorrhage, the slow healing, the complications of blood poisoning, erysipelas, gangrene and tetanus, the physical and mental anguish, and the uncertainty of the final outcome. The mortality from major operations was from 50 to 100 per cent.'

"Today, on the contrary, the opening of the abdomen, the chest or the skull no longer is equivalent to signing the death warrant of the patient. Pasteur proved that fermentation and putrefaction were neither spontaneous, on the one hand, nor due to occult causes, on the other, but are in reality the result of the activity of minute living organisms. Among the fruits of Pasteur's labors was the work of Joseph Lister.

"It is sometimes stated that antiseptic surgery had its birth in 1867, when Lister reported, in the *Lancet*, his eleven cases of compound fracture, with a 'prelim. note' on the antiseptic method of opening abscesses. He had furnished the first solution of the problem of how to prevent putrefaction in open wounds. As Paget⁵ remarks, 'Pasteur could prevent putrefaction in broth, by his aseptic method: but patients cannot be boiled, nor kept in filtered air, in flasks.' To kill the germs in the wound, Lister at first chose phenol (carbolic acid). To prevent any more germs from getting in, he left untouched the scab or crust formed by the antiseptic and blood together on the wound. His first case, in Mar., 1865, failed; his next case, in Aug., was successful.

"Half a century has elapsed since those memorable experiences.

⁴ Lee: Scientific features of modern medicine, Columbia Univ. Press, N. Y., 1911.

⁵ Paget: Pasteur and after Pasteur, 1914, p. 36.

The wonderful strides which surgery has made during these years are fresh in our minds. The work of the surgeon is not confined to the repair of wounds, the correction of deformities, or the removal of tissues. Deficiencies may be supplied by transplantation; transfusion is readily carried out; a new era in reconstructive surgery has been inaugurated. There is no reason to believe that the end of these progressive advances in surgery in recent years is in sight. As a recent writer has remarked, surgery is no longer merely an art of skilful cutting and sewing; it has risen to the higher level of a science.

“The incalculable benefit of Lister’s studies, and of that which has grown out of them, can best be appreciated in the contemplation of the surgical infections which, in the memory of physicians now living; were once the dread of all operating surgeons. Park⁶ has thus recorded his impressions of the earlier days: ‘I deem myself fortunate in this—that I have been a living witness of the benefit of the change from the old to the new, since when I began my work, in 1876 (over twenty years ago), as a hosp. intern, in one of the largest hosp. in this country, it happened that during my first winter’s experience—with but one or two exceptions—every patient operated upon in that hosp., and that by men who were esteemed the peers of any one in their day, died of blood poisoning, while I myself nearly perished from the same disease. This was in an absolutely new building, where expenditure had been lavish; one whose walls were not reeking with germs, as is the case yet in many of the old and well-established institutions. With the introduction of the antiseptic method, during the two years following, this frightful mortality was reduced to the average of the day, and in the same institution today is done as good work as that seen anywhere. The same was true without exception in the great hospitals of the Old World; and in Paris, where, 30 years ago, famous surgeons would go from one end of the building to the other, handling one patient after another without ever washing their hands, and where erysipelas and contagion of various kinds were thoroughly distributed, as it were, impartially, now the successors of these very same men, employing modern methods, get results which challenge comparison.’

⁶ Park: An epitome of the history of medicine, 1898, p. 326.

“Those who fail to recall the historical development of the modern surgical technic are sometimes wont to assume that because certain current *aseptic* procedures have entirely superseded the *antiseptic* devices introduced by Lister, the advantages of his precautions are no longer essential. It must be remembered, however, that antiseptic and aseptic are nothing other than two ways of arriving at one result. In the words of Sir William Osler: ‘It is the difference between tweedledum and tweedledee. They are both applications of the same principle.’ We cannot summarize the situation better than by again quoting Paget: ‘It is true,’ he writes, ‘that Lister was more afraid of the powers of the air, half a century ago, than surgeons are now; it is true that his use of a phenol spray, to sterilize the air around the wound, has been given up; but the law of all operations remains today that law which was revealed to Lister in 1865, in the light of Pasteur’s work on putrefaction. From the very first, antiseptic surgery had in itself the making of aseptic surgery.’

“The principles defended and applied by Lister find applications in everyday life at the hands of the layman; their value is further attested in the prominence which they play in the modern military régime. The soldier on the European battlefield has been taught to apply an antiseptic dressing promptly to the wound he receives in action, and the enlightened teachings of Lister follow him through all the subsequent stages of his treatment to recovery. After a lapse of 50 years we may well pause to recall that Lister was instrumental in saving more lives than the armies of the greatest general or potentate have destroyed. Let us mark the anniversary on our medical calendar and pay a tribute to the masterful genius of Lister, ‘a man serene through controversy, a spirit of invincible patience and of radiant purity.’” EDITORIAL: *Jour. Amer. Med. Assoc.*, 1915, lxxv, p. 171.

II. WAR NOTES

Necrology.—Dr. *Chaillou*, head of the anti-rabies dep’t of the Pasteur Inst.

G. C. M. Mathison, known for his work on the physiol. of respiration.

Max Rappart, assis. in chem. to Fischer, Univ. of Berlin.

E. Rhodé, instr. in the physiol. and pharmacol. of the heart,

Univ. of Heidelberg; died of pneumonia while engaged in Red-Cross service.

Hugo Lüthje, chief of the Med. Clinic, Kiel Univ., author of exhaustive studies of nutritional diseases; died of typhus contracted during a visit of inspection to a camp of Russian prisoners.

University items. Dr. *V. E. Henderson*, prof. of pharm. and pharmacol., Univ. of Toronto, commands the detention camp at Kapuskasing, Ontario.

Among the British scientific men in military service are Dr. *J. A. Gunn* (reader in pharmacol., Oxford), Lieut. R.A.M.C., and Sir *Wm. Osler* (regius prof. of med. Oxford), Hon. Col. S. Midland Div. R.A.M.C.

Dr. *J. George Adami* (prof. of pathol., McGill Univ.), is now serving as a member of the British War Office, having charge of the preparation of a medical history of the war.

Booze bombs. PROHIBITION OF ALCOHOLIC DRINKS IN THE FRENCH ARMY. The example set months ago by Russia, on the "liquor question," has been followed by other nations. The following note relates to conditions in France. "An order of Gen. Galliéni, mil. gov. of Paris, forbidding the sale of alcoholic drinks to soldiers garrisoning the defences of Paris, was published in July. Now, when the physical and moral energy of soldiers ought to be carried to their highest degree of intensity, the order explains, it is important that the campaign against alcohol, which destroys both, should be carried on without faltering. Hence it is forbidden to sell alcohol and alcoholic drinks (absinthe, vermouth, bitters, apéritifs, liqueurs, etc.) to soldiers of any grades. Liquor sellers will have their establishments closed temporarily for the first offense and permanently for the second." PARIS LETTER: *Jour. Amer. Med. Assoc.*, 1915, lxx, p. 636.

ON BEER AND BRANDY IN THE GERMAN ARMY. "In the *Journal (Amer. Med. Assoc.)*, May 15, 1915, p. 1663, appears editorial comment entitled 'Alcohol in the European Armies,' which is based on figures obtained from the *British Med. Jour.* discussing the daily consumption of alcohol by the armies in the field. So far as these figures are intended to apply to the German troops, they are without basis and fact. It is said, in the edit. comment, that the German

soldier is allowed 1,793 gm. of beer and 20 gm. of brandy daily, amounting to a total of 70.7 gm. of alcohol a day. I do not know how you or the *British Med. Jour.* happened to take the figure 1,793 gm. of beer, but that this could not possibly be true should have occurred to you. Let us assume, for the sake of argument, that there are 3,000,000 soldiers at the front, although there are probably more than that. They would receive 5,379,000 kg. of beer daily. A liter of beer, including packing, weighs, according to the estimation of the breweries, 1.65 kg., so that the daily allowance for the army would total 8,075,350 kg. The ordinary freight car carrying beer will hold 10,000 kg. Therefore, 887 such cars filled with beer would have to be shipped to the front daily and as many returned. Such freight traffic, under present conditions, is an impossibility because so many other things must be transported to the front at this time. *In reality there is no regular alcohol allowance or consumption.* The soldiers are given coffee and tea as stimulants, and *not alcohol*; and if, by any chance, a keg of beer should happen to fall into the hands of the soldiers, it would be cause for celebration. You must be prepared to find unsolved in the final analysis the problem as to how much military efficiency among the German troops may be increased by means of so-called 'Dutch courage.'"

Very respectfully yours,

WALTHER STRAUB, M.D.,

Professor of pharmacology, University of Freiburg in Breisgau,

July 10, 1915

(*Jour. Amer. Med. Assoc.*, 1915, lxx, p. 732).

Honors. The Paris Acad. of Sciences, in secret session, passed a resolution removing from its membership four German scientific men, including Emil Fischer.

Prof. Roentgen was 70 years old, Mar. 27. In honor of the occasion the Kaiser presented him with an Iron Cross. In the accompanying message of congratulation, the Kaiser said: "The German nation cannot be grateful enough to the discoverer of the rays for whom they are named. The many advantages from the use of the rays are being rendered apparent by the war more than ever before."

Sir Wm. Ramsay, recently writing on German "Kultur," shows that of the 58 awards made during the past 12 years by the Swedish Nobel Commit., only 17, or merely 30 percent, were received by Germans and Austrians. The ratio of German and Austrian foreign members and associates of the principal academies of the world is only 28 percent.

"Our foreign exchanges now contain an occasional article with the subheading, 'Dedicated to Elias Metchnikoff on the occasion of his 70th birthday, May 16, 1915.' These articles had been prepared for an international *Festschrift*, which was to have been presented to him on that date, but the war broke up the plans for the volume, and the contributions are being published separately in various neutral journals. He is a native of southern Russia but has had a lab. at the Paris Pasteur Inst. since its erection, over 27 years ago. His discoveries in phagocytosis were made previously in Italy. Although he studied under German physicians, he never took a degree in medicine." *Jour. Amer. Med. Assoc.*, 1915, lxiv, p. 2080. (See page 246.)

Notes on the sanitation of military quarters and battlefields. Dr. F. Bordas, substitute prof., Coll. de France, has suggested to the Acad. des sciences that the disinfection of cantonments, trenches occupied by troops, and battlefields, be effected by copious spraying with petroleum emulsified in water by means of a suitable amount of rosin soap.

"In the present war, in which the battle fronts extend in France and Belgium alone over more than 375 miles, the question of the sanitation of battlefields has a special importance. . . . In general, the use of chemical agents and spray applications (coal tar, phenol, ferrous sulfate, zinc sulfate, chlorinated lime, etc.), is recommended. These chemical agents, however, are rather expensive and it is not always possible to obtain them. It is therefore of interest to recall that, in 1871, the Conseil d'hygiene publique recommended, and applied with success, an altogether different method, based on the power possessed by vegetable growths of absorbing and transforming decaying animal substances. By this method bodies are left where they fall, but covered with a layer of earth no thicker than about 15 to 20 inches. It is necessary, however, to take care to bring the

earth for the mound from a distance of about 1 to 2 yards, in order not to disturb the soil impregnated with liquids of decomposition and to avoid forming trenches at the side which would dry the mound too rapidly. The mound is then planted with grains and plants that grow rapidly and which are especially avid of nitrogen or the products of decomposition. Among the best for this use are the *Helianthus annuus* or common sunflower and the *Balsamita suaveolens*, otherwise known as the *Chrysanthemum balsamita* or balsam herb, whose assimilative powers are remarkable. Other plants that are very greedy for nitrogen may be employed, such as the *Galega officinalis* or goat's rue, the *Helianthus tuberosus* or Jerusalem artichoke, the *Sinapis arvensis* or wild mustard, and some forage plants." PARIS LETTER: *Jour. Amer. Med. Assoc.*, 1915, lxv, p. 813.

Food notes. CONDITIONS IN MEXICO. Distressing economic conditions prevail in Mexico. Mr. C. J. O'Connor, the repr. of the Amer. Red Cross in Mexico City, reports that the amount of food necessary for an individual per day costs at present nearly \$10 Mexican, while the laborer's wage is \$0.75 Mexican. Civilians in Saltillo are reported to be eating donkey flesh and cactus. No corn or beans can be purchased at any price.

THE FOOD SUPPLY IN GERMANY. "The restrictions that were imposed on the consumption of bread are fortunately being reduced more and more. When it was learned officially that there is a large reserve of grains to carry us to the harvest (7 millionen *Doppelzentnern Getreide*), the bread ration per capita was increased. The authorities regarded it as especially important that the bread supply for working people should be made more ample. However, every one soon became accustomed to the restrictions on bread, and no one complains any more. This is the more readily understood as the consumption of meat and of bread used to be above what was actually required—a *Luxuskonsumtion*. With regard to other foods, conditions have righted themselves so that the menus show scarcely any difference from those of peace times. The only thing in this line that reminds us of the war is the increased cost of meat and other articles of food except certain vegetables. It is remarkable, moreover, that other countries, even the neutral ones, are hav-

ing a similar experience, suffering from higher prices to almost the same degree as we are.

“Some official figures have been published recently comparing the prices now and a year ago:

1 kg. wheat flour,	May, 1914,	0.37 mark;	May, 1915,	0.55 mark
1 kg. rye flour,	“	0.29 “	“	0.55 “
1 kg. potatoes,	“	0.07 “	“	0.15 “
1 kg. butter,	“	2.61 “	“	3.54 “
1 kg. milk,	“	0.20 “	“	0.24 “
1 — egg,	“	0.07 “	“	0.11 “
1 kg. sugar,	“	0.50 “	“	0.58 “
1 kg. coffee,	“	3.08 “	“	3.35 “
1 kg. rice,	“	0.48 “	“	1.22 “

“It should be added that the prices in May were in part lower than in the preceding months. In London, according to the London corresp. of the Swiss Zurich *Post*, flour is 71 percent higher than last year; butter, 24 percent; meat, 30 percent; tea, 25 percent; sugar, 88 percent; fish, 50 or 75 percent; refrigerated meat, 100 percent.

“Naturally, the increased cost of living would bear particularly hard on the poorer classes, especially those families whose breadwinner is serving in the army, if it were not for the assistance rendered by the state, local organizations and private philanthropy. Without exaggeration I can affirm that of my own knowledge I am not aware of any distress from the high cost of living at present more than in times of peace, and the lay press has not published anything suggesting this in any part of the empire. My assertion will receive more credence when the amount of the sums that have been contributed for assistance is realized. . . . The social insurance companies had spent for relief purposes, in connection with the war, up to June 1, \$3,250,000 of their reserve funds. Besides this, \$14,000,000 have been loaned to communities for relief purposes, and they took up \$72,500,000 of the war loans.” BERLIN LETTER: *Jour. Amer. Med. Assoc.*, 1915, lxxv, p. 441.

THE FEEDING OF PRISONERS OF WAR IN GERMANY. “The War Dep’t recently organized a course of instruction for the officials in

charge of the concentr. camps of war prisoners. The chief aim of the course was to instruct the officials in the essential principles of the physiol. of nutrition and the art of cooking. The officials came from 129 different concentr. camps, from all over Germany, and leading specialists delivered lectures on the scientific bases for, and the practical features of, the feeding of the prisoners. Dr. Neumann, direc. of the inst., at Berlin, for research on the utilization of grains, spoke on the essentials of bread as food. This is a particularly difficult subject, as the bread-ration has had to be reduced for the prisoners as well as for the civilian populace. As the French and Russians are accustomed to make much use of bread, the Minister of War had large amounts of second-grade rye and wheat, which were not suitable alone for making bread, mixed with other kinds of flour. This mixed flour was used to bake a supplementary bread-supply for the concentr. camps, where it was sold to the prisoners through the canteens for 50 pfennigs per kg. or, as rolls or cakes, by weight, 75 gm. for 5 pfennigs. It can also be supplied gratuitously, on a physician's order, to certain undernourished persons and certain others engaged in manual labor. The standard dietary suggested by the Minister of War is, for every prisoner of war, military or civilian, 85 gm. protein, 40 gm. fat and 475 gm. carbohydrate, a total of 2,700 calories. Those who have to do manual labor, particularly the inmates of the work-camps, are to receive 10 percent more. This ration is to be given in three nourishing and palatable meals: in the morning, coffee with 30 gm. of sugar, or a soup containing 100 gm. of solids. A soup has proved particularly satisfactory that is made of 30 gm. soy beans, 60 gm. flour (*Stärkemehl*) and 10 gm. fat. The dinner is to consist of 750 gm. potatoes, and 300 gm. fresh or canned vegetables or 40 gm. dried vegetables. Every third day meat is to be given with the above. It was recommended to give fresh meat twice to corned meat once. The amount is prescribed as 120 gm. meat with bones or 100 gm. without bones. On the two intervening days, 200 gm. fish is recommended or 150 gm. pod vegetables, with the addition of bacon, fat or pickled meat. Soy beans in the form of flour were particularly recommended. Salt, spices and fat are not to be spared. For supper, baked potatoes with herring or sausage or cheese have

proved satisfactory; the potatoes can be given in the form of a salad. Thick soups made from beans or flour have also proved useful for supper. Rice with baked or stewed fruit may also be used. Opportunity for tea drinking is given the Russians. Where skim-milk is obtainable, it shall be supplied abundantly to the prisoners. It was emphasized that the tastes of the prisoners should be considered as much as possible in the selection and preparation of the dishes." BERLIN LETTER: *Jour. Amer. Med. Assoc.*, 1915, lxxv, p. 544.

Medical notes. MEDICAL-SUPPLY EXPORTS DOUBLED. The U. S. Bur. of Foreign and Domestic Commerce has estimated that during the year ended June 30, 1915, the exports of medicines and surgical instruments amounted to \$35,744,000 as compared with \$19,916,000 for the preceding year.

WAR HELPS MEDICAL WOMEN IN ENGLAND. A statement signed by Premier Asquith, Lord Curzon, and Hon. A. J. Balfour calls the attention of the British public to the work in London of the School of Med. for Women, which has now doubled its plant in an endeavor to cope with the war-time increase of opportunities for women physicians. The statement begins as follows: "*The war constitutes the turning point in the position of medical women, for whom there are new openings and new opportunities in many directions.*"

MEDICINAL PLANTS IN GERMANY. The *Münch. med. Woch.* states that the German Minister of the Interior has appealed to the apothecaries to stimulate the collection and drying of medicinal plants and parts of plants in their districts. They can then prepare them for med. use, each in his own laboratory or by exchanging them with others. He explains that large amounts of the plant-drugs used in making medicines have always hitherto been imported from other countries for this purpose. The war has rendered it very difficult to import them now or has shut off the supply completely. A list of plants, useful for the purpose, is given, including the flowers of arnica, chamomile, linden, elder and mallow, and the leaves of digitalis, walnut, belladonna, colt's-foot, henbane, stramony, buck-bean, and various herbs and berries. Children, it is urged, can be taught to collect them, and also the elderly and the otherwise

incapacitated, so that there need be no difficulty in collecting an adequate supply.

RAPID WHITENING OF THE HAIR AFTER EXPLOSION OF A MINE. "At one of the recent sessions of the Société med. des hôpitaux, Paris, Dr. Lebar reported the case of a soldier, aged 33, who, having been blown into the air by the explosion of a mine, next day had locks of white hair on the left side of his head. The decoloration of the hairs was complete from base to extremity. The longest as well as the shortest were white and there was not a brown one among them. All the hairs that became suddenly white are still firmly implanted. It was suggested that the general nervous shock caused by the explosion of the mine set in motion the medullary cells of the hair, the pigmentophagic rôle of which has been shown by Metchnikoff." PARIS LETTER: *Jour. Amer. Med. Assoc.*, 1915, lxxv, p. 183.

THE SO-CALLED NEW ANTISEPTIC. "Recently the newspapers have contained announcements of a new antiseptic or germicide that has proved, or is to prove, of great value in the treatment of the wounded in the present war. Credit for its discovery is given to Drs. Carrel and Dakin.

"The antiseptic referred to is that which Dr. Dakin,⁷ of the Herter Laboratory, N. Y. City—now serving as bacteriologist in a war hosp. at Compiègne, France—announced in a paper read before the Acad. des sciences, Paris. It is made by the well-known process of adding sodium carbonate to a sol. of chlorinated lime. The mixture is thoroughly shaken, and after half an hour the liquid is siphoned off from the precipitate of calcium carbonate and filtered through cotton. To this clear liquid, sufficient boric acid is added to make the preparation neutral or acid, the amount required being determined by titration with phenolphthalein. Such a sol. was found to kill staphylococci in two hours.

"According to the *British Med. Jour.*,⁸ about a year ago Prof. Cohen, of the Univ. of Leeds, entered into communication with Dr. Dakin, a former student, regarding research on antiseptics for surgical use. The arrangement was that the substances elaborated

⁷ Dakin: *Presse méd.* (society proceedings), Aug. 5, 1915.

⁸ Research in Antiseptics, *Brit. Med. Jour.*, Aug. 14, 1915, p. 261.

by Prof. Cohen should be tested bacteriologically by Dr. Dakin, and that the most promising should be tried clinically by Dr. Carrel.

"At about the same time, under the auspices of the English med. research commit., a similar research by Prof. Lorrain Smith, with the assistance of Prof. Drennan of the Univ. of Otago, N. Z., Dr. Rettie, a chemical expert, and Lieut. W. Campbell of the British army med. corps, was undertaken in the Univ. of Edinburgh. Their results were reported in the *British Med. Jour.*⁹ The substance which they prepared was made by rubbing chlorinated lime to a fine powder and mixing it with an equal weight of powdered boric acid. The ideal antiseptic for the field, they concluded, was a dry powder to be applied direct, which, it was believed, has advantage over a sol. because it is more portable, and water is often not procurable.

"Chlorinated lime, the basis of the so-called new antiseptic preparation, is well known as a powerful disinfectant. Its alkalinity, however, makes it destructive to living tissues except in dilute sol. The same may be said of sol. of chlorinated potash (Javelle water), which has been largely used by French surgeons in the present war, and of sol. of chlorinated soda (Labarraque's sol.). The advantage claimed for the new mixture is that the preparation, being practically neutral and unirritating to the tissues, may be applied in greater strength than that in which it is possible to use chlorinated lime, Javelle water or Labarraque's sol. Experiments indicate also that the germicidal activity of chlorinated lime is increased by such treatment of the calcium hypochlorite as has been described. Such increase in germicidal activity is generally attributed to the liberation of hypochlorous acid. It has been found that the activity of ordinary bleaching powder is greatly increased by passing through it carbonic acid gas. Any other acid, as boric acid, will do as well.

"From the chem. point of view, therefore, there is nothing new in this method. That the practical application of such a mixture is not wholly new is proved by an earlier article published by Vincent.¹⁰ He suggested the application to ulcerating and gangrenous wounds

⁹ *Brit. Med. Jour.*, July 24, 1915, p. 129; abstr., *Jour. Amer. Med. Assoc.*, Aug. 21, 1915, p. 744.

¹⁰ Vincent: *Presse méd.*, 1914, xxii, No. 70; abstr., *Jour. Amer. Med. Assoc.*, Nov. 28, 1914, p. 1986.

of a mixture of chlorinated lime and boric acid. EDITORIAL: *Jour. Amer. Med. Assoc.*, 1915, lxxv, p. 880.

Miscellaneous items. "*Chemical Abstracts* will be the only complete record of chemical research reported during the war period." E. J. Crane: *J. Ind. and Eng. Chem.*, 1915, vii, p. 465.

A deputation from the Royal Soc. and the Chem. Soc. was received by the pres't of the Boards of Trade and Educ., in London, May 6. The dep. was introduced by Sir Wm. Crookes, pres't of the Royal Soc. Prof. W. H. Perkins, Sir Wm. Tilden, Prof. P. Frankland, Prof. W. J. Pope and Dr. M. O. Forster spoke in support of memorials from the two societies, indicating the steps which might be taken immediately to improve the status and efficiency of the chemical industries and those engaged in them in the United Kingdom.

III. COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

I. General notes

Honors. HON. DEGREES. Drs. *R. H. Chittenden* and *S. J. Meltzer* were among the distinguished biologists who received the hon. degree of LL.D. on the occasion (Apr. 30) of the dedication of the new buildings of Washington Univ. Med. Sch., St. Louis.

MEDALS. At the last annual commencement of the N. Y. Coll. of Dental and Oral Surgery (June 8), Dr. *Louise C. Ball* was the recipient of three medals and one "honorable mention," as follows: *Clarkson Cowl Gold Medal* (highest award), for the best average stand in the final exam's in the full course for three successive years in the Coll.; *Operative Dentistry Medal*, for the highest mark in the final exam. in operative dentistry; *Chemistry Medal*, for the best thesis in dental chemistry; *Sanger Medal*, first hon. mention (second in rank), for the best work in theoretical and practical prosthetic dentistry, during three successive years in the Coll. At the commencement in 1914, Dr. Ball received the only medals offered to juniors: *Faculty Medal*, for the highest marks in the final exam's of the jr. year; *Oral Surgery Medal*, for the best thesis on suppuration. Dr. Ball is vice-pres. of the class of 1915, which has 77 members.

SILLMAN BIOCHEM. SOC. Members of the soph. class, Coll. of

Med., Baylor Univ., have organized the *Sillman Biochemical Society*, which is named in honor of Prof. *Maxwell Sillman*. The society's object is the study of the relationships between the med. sciences and chemistry, and to stimulate biochem. investigation in general. The officers for the next academic year are: Pres., *W. W. Looney*; vice-p., *H. L. Farmer*; sec-treas., *W. N. Bunkley*.

Appointments.¹¹ Albany Med. Coll.: *Arthur Knudson*, assis. prof., biol. chem. (prom.).

Coll. City New York: *L. J. Curtman*, assis. prof., chem. (prom.).

Fordham Univ., Med. Sch.: *D. R. Lucas*, lab. assis., physiol.

Iowa State Coll. (Ames): *Helen Monsch*, head, Dep't of Foods.

N. Y. State Dep't of Health (Albany), Div. of Lab's and Research: *Tula L. Harkey* (Nat'l Path. Lab., N. Y. City), lab. assis., bacteriol.

Rockefeller Inst. Med. Research: *M. Heidelberger*, assoc., chem. (prom.).

Univ., Cal.: *H. A. Mattill* (Univ., Utah), assis. prof., nutrition.

Univ., Neb., Coll. of Med. (Omaha): *Max Morse* (Univ., Wis.), assis. prof., biochem.

Vassar Coll.: *Cora J. Beckwith*, assoc. prof., zoology (prom.).

Va. Agric. Exp. Sta'n (Blacksburg): *F. D. Fromme* (Purdue Univ., Agric. Exp. Sta'n), plant pathologist and bacteriologist.

West. Maryland Coll. (Westminster): *P. W. Punnett*, prof., chem. and biol.

Dental graduates. At the recent commencement of the N. Y. Coll. of Dental and Oral Surgery, the degree of D.D.S. was conferred on *Louise C. Ball* and *Siegfried J. Nilson*.

Associations and societies.¹² OFFICERS-ELECT. Medical Brotherhood: Pres., *S. J. Meltzer*; councilor, *N. B. Foster*; hon. pres., *R. H. Chittenden*; members of advis. commit., *C. L. Alsberg*, *John Howland*, *L. B. Mendel*.

Sigma Xi (Yale Chapt.): *L. L. Woodruff*, pres.

MEMBERS-ELECT. Amer. Philosoph. Soc.: *P. B. Hawk*.

Nat'l Acad. Sciences: *Alexander Smith*.

Sigma Xi (Columbia Chapt.): *Louise H. Gregory*, *E. G. Griffin*,

¹¹ See also page 456.

¹² See also page 457.

J. H. Northrup, H. H. Plough, George Scatchard, A. P. Tanberg, J. R. Tuttle.

Soc. Exp. Biol. and Med.: *George M. Bachr, D. J. Edwards.*

Miscellaneous items. In his capacity as sec'y of the Assoc. of Off. Agric. Chem., Dr. *C. L. Alsberg* is a member of the ed. commit. of the newly founded *Journal* of that Assoc.

Dr. *Geo. D. Beal* is one of the members of the ed. board of *The Register* of Phi Lambda Upsilon; also of *The Illinois Chemist*.

Dr. *G. Delgado Palacios*, prof. of pathol. chem., Univ. of Caracas and member of the Venezuelan Acad. of Med., is a visitor in this country and temporarily engaged here in chemical work. Address: 118 East 116th St., N. Y. City. See page 278.

The Texas Public Health Assoc. has appointed Dr. *L. B. Bibb*, of Austin, to secure the coöperation of other agencies in the State to undertake a thorough experimental investigation of the value of cottonseed meal as human food. Miss *Anna E. Richardson*, of the Domestic Economy Dep't, Univ. of Texas, has been appointed a member of this Commit., and, with the coöperation of Miss *Helen S. Green*, has undertaken to investigate the nutritive value of cottonseed meal. The work is being carried on by metabolism experiments and extensive feeding of small animals.

Dr. *F. C. Phillips*, emer. prof. of chem., Univ. of Pittsburgh, was recently the guest of his pupils at a testimonial dinner, in Pittsburgh, when he was presented by them with a gift of \$1,000. The movement that culminated in this cordial expression of affection for Prof. Phillips was inaugurated by Dr. *Jacob Rosenbloom* during his term of office in the Columbia Biochem. Dep't.

The directors of the Retail Drygoods Assoc. of N. Y. City have voted to establish a Commit. on Health and Sanitation, to coöperate with the N. Y. Dep't of Health in protecting the health of the 50,000 employees of the retail drygoods stores in N. Y. City. The owners of large department stores are planning to secure and maintain the best working conditions for those in their employ. The Bur. of Public-Health Educ., under the direction of Dr. *C. F. Bolduan*, will establish a course of lectures and a publicity campaign for the purpose of teaching employees how to be sanitary and healthy.

2. Proceedings of the Association

Edgar G. Miller, Jr., Secretary

Twenty-third (sixth annual) meeting. The concluding quarterly meeting of the Assoc., for 1914-'15, was held in the Library of the Columbia Med. Sch., May 28, at 8 p. m. This meeting followed an informal dinner at 6.30 in the same room, which was greatly enjoyed by all in attendance.

The feature of the program was Dr. *C. F. Bolduan's* interesting discussion of the "Educational lunch room," conducted by the Bur. of Public-Health Educ. of the Dep't of Health, N. Y. City, of which Dr. Bolduan is director. Abstracts of the papers comprising the biochem. proc. will be published in the next number of the *BIOCHEM. BULL.* The matters of public interest in the exec. proc. are noted below.

The commit. "to endeavor to quicken the interest of Amer. med. men" in the proposed "*Medical Brotherhood*," organization of which was suggested by Dr. Meltzer at the fourth annual dinner (pages 263, 267 and 292), was appointed as follows: S. J. Meltzer, chairman; Carl L. Alsberg, Nellis B. Foster, Wm. J. Gies, A. J. Goldfarb, Alfred F. Hess, Lafayette B. Mendel.

The time for the recurrent annual meeting was changed from that of the last regular meeting of the *academic* year to that of the last regular meeting of the *calendar* year.

It was voted to reëlect the present officers, to serve until the election of their successors at the regular Dec. meeting. (The list of officers was printed on page 228.)

The register of members of the Assoc., as published by the sec'y in No. 13 of the *BIOCHEM. BULL.*, was made the official role of membership to date.

Miss *Hattie L. Heft* was unanimously elected assis. sec'y for the term ending Dec., 1915.

The next regular meeting of the Assoc. will be held on Dec. 3.

3. Columbia Biochemical Department

Appointments. FROM THE STAFF. Dr. *Arthur D. Emmett*, instr. in this dep't during the past year, has been reappointed assis. chief, animal nutr., Agric. Exp. Station, Univ. of Ill., Urbana.

FROM THE BODY OF ADVANCED STUDENTS. Amer. Mus. Nat. Hist., Dep't Public Health (N. Y. City): *I. J. Kligler*, scientific assis., in charge of the lab. of bacteriol. (part time).

Columbia Univ., Sch. of Med.: *J. Howard Mueller*, Alonzo Clark Scholar.

Cornell Univ. (Ithaca, N. Y.): *Mary F. Henry*, instr., home economics.

Lederle Lab. (N. Y. City): *I. J. Kligler*, assis., bacteriol. and biochem. (part time).

Montana Agric. Coll.: *Lilla A. Harkins*, prof., domes. science; head of the dep't.

Mt. Holyoke Coll. (So. Hadley, Mass.): *Anna B. Yates*, instr., physiol.

Ottawa High Sch. (Ill.): *Isabel Clegg*, head, dep't of househ. arts.

Penn. Hosp. (Phila.): *Mrs. Jennie D. Wood*, head dietician.

Rockefeller Inst. Med. Research: *G. E. Cullen*, assis., chem. (prom.).

Trenton Public Sch. (N. J.): *Jennie P. Case*, supervisor, domes. art and science.

Univ., Minn.: *Lucile Wheeler*, assis. prof., foods and cookery.

Appointments to the staff. *L. H. Almy* (U. S. Food-Research Lab., Phila.), univ. scholar, biol. chem.

Arnold K. Balls (U. S. Bur. of Chem.), assis., biochem.

Adolph Bernard (N. Y. Post Grad. Med. Sch.), univ. scholar, biol. chem.

Frederick G. Goodridge, assoc., biochem. (prom.).

Sergius Morgulis (U. S. Bur. of Fisheries; resident in this lab., 1914-'15), reappointed to the instructorship held 1913-'14.

Associations and societies. Amer. Pharmaceut. Assoc.: *V. E. Levine*, member.

Amer. Philosoph. Soc.: *Wm. J. Gies*, member.

Medical Brotherhood: *Wm. J. Gies*, first sec'y and member of the Exec. Com.

Soc. Exp. Biol. and Med.: *Wm. J. Gies*, member of the Council.

Addresses, lecture and reports. Prof. *Gies* was one of the speakers at the 16th ann. dinner of the Alumni Assoc. of the Coll.

of Dental and Oral Surgery of N. Y., at the Hotel Manhattan, April 17. He delivered the lecture at the annual joint session of Sigma Xi and Phi Beta Kappa, at Columbia Univ., May 19, on Diseases of the teeth and bones, their causes and prevention, with some demonstrations.

Prof. *Gies* attended the 46th annual meeting of the Dental Society of the State of N. Y., in Albany, May 14, and there presented a report, in collaboration with *E. G. Miller* and *W. A. Perlzweig*, on the results of research on the relation of internal secretions and diet to dentition. He presented to the 14th Internat. Lord's Day Congr., Oakland, Cal., July 28, a report, in collaboration with *A. D. Emmett* and *Katherine R. Coleman*, on the results of research on the physiological influence of a recurrent weekly day of rest, as measured in terms of effects on general nutrition. Dr. *Gies's* duties during the summer session made it impossible for him to present the report in person.

Awards of higher degrees at Columbia to students of biolog. chem. DOCTORS OF PHILOSOPHY. Of the 28 recipients of the degree of Ph.D. under the Fac. of Pure Science, at Columbia's last commencement, 10 had taken "majors" or "minors," or both (or "extra" advanced courses) in the Biochem. Dep't. The names of the candidates, and the subjects of their major and minor courses, are given below.

Name of candidate	Major	Minor	Minor
Arthur D. Emmett	biol. chem.	chem., food	bacteriology
Frederick G. Goodridge	biol. chem.	biol. chem.	pharmacology
Edward G. Griffin	chem., org.	chem., phys.	biol. chem.
Mildred A. Hoge	zoology	zoology	{ physiology
Roscoe R. Hyde	zoology	bacteriology	{ biol. chem.
Israel J. Kligler	biol. chem.	bacteriology	{ bacteriology
Alexander Lowy	chem., elec.	chem., org.	{ pathology
Percy W. Punnett	chemistry	chemistry	{ biol. chem.
Arthur P. Tanberg	chemistry	chemistry	{ education
Arthur W. S. Thomas	chemistry	chem., inorg.	{ biol. chem.
			{ biol. chem.

MASTERS OF ARTS. The A.M. degree was recently conferred upon the following advanced students in the Biochem. Dep't: *A. K. Apisdorf*, *J. C. Baker*, *O. C. Bowes*, *H. B. Clough*, *Helen C. Coombs*, *Hazel Donham*, *W. J. Donvan*, *Jessie V. Farr*, *Helen G. Gates*, *Lucy*

H. Gillett, Helen S. Green, C. P. Harris, F. W. Hartwell, Hattie L. Heft, Jacob Hoffmann, Lottie M. Hull, Mabel M. Lutes, Marguerite L. McLean, Jeannette C. Mullikin, Alma M. Oswald, Almeda Perry, H. H. Plough, Helene M. Pope, W. H. Schliffer, Jr., J. J. Tanzola, M. K. Thornton, Jr., Lucile Wheeler, Anna B. Yates.

DOCTORS OF PHARMACY. The following students of biol. chem. at the N. Y. Coll. of Pharm. received the degree of Phar.D.: José E. Argüello, S. E. Posin.

Summer session. COURSES. The Dep't conducted five courses in nutrition, biochem. methods, and research, during the recent summer session (July 6–Aug. 14). Two of these courses were given at Teachers Coll., by Prof. *Gies*, Dr. *Emily C. Scaman* and Miss *Helen C. Coombs*; three were given at the Coll. of Phys. and Surg. by Prof. *Gies* and Mr. *W. A. Perlzweig*. The biochem. lab. at the Med. Sch. was open daily for research throughout the summer.

INVESTIGATORS. The workers named below were engaged in research, in the biochem. lab. at the Med. Sch., at various times during the summer vacation:

B. Aronowitch, Robert Bersohn, O. C. Bowes, *Katherine R. Coleman*, A. D. Emmett, Wm. J. Gies, B. Horowitz, C. H. Jordan, I. J. Kligler, Arthur Knudson, S. Kubushiro, V. E. Levine, F. Lowenfels, E. G. Miller, Jr., Sergius Morgulis, A. Mutscheller, Wm. A. Perlzweig, Louis Pine, G. J. Rosenthal, Maxwell Sillman, J. R. Tuttle, Wm. Weinberger.

Miscellaneous items. Dr. *A. D. Emmett* is one of the three members of the ed. board of *The Register* of Phi Lambda Upsilon.

Dr. *Benjamin Horowitz* was one of the delegates from the Collegiate Zionist League to the Zionist Convention in Boston, June 27–July 1.

Dr. *Gies* recently served as chairman of a sub-committee of the Commit. on Food Inspection of the Advisory Council of the N. Y. City Dep't of Health, to "deal with the problem of the use of copper tanks by candy manufacturers."

EDITORIAL

WILLIAM J. GIES

The opening pages of this issue of the *BIOCHEMICAL BULLETIN* publish the notable address by Dr. Meltzer, in which he proposed the organization of a *Medical Brotherhood for the Furtherance of International Morality*. At page 292 of this issue we present, also, a general statement regarding the "origin, organization and proceedings" of the Medical Brotherhood (prior to Oct. 1).

Although the Medical Brotherhood is not a biochemical organization, we give its affairs a prominent place in this issue because the organization of the Medical Brotherhood was proposed by a biochemist at a biochemical dinner, was endorsed at a subsequent biochemical meeting, includes in the membership of its Executive and Advisory Committees four past presidents and two past secretaries of the American Society of Biological Chemists, numbers among its members many biological chemists, *invites all biochemists to rally to its standard*, and deserves universal support and encouragement.

The official invitations to membership require (as the only condition of membership for those who are eligible to election) endorsement, by signature, of the following avowal:

"I am in full sympathy with the sentiments expressed in the Appeal (p. 300), and desire to be enrolled as a member of the Medical Brotherhood (*Fraternitas medicorum* = **F.M.**)."

The official announcements pertaining to membership also include the following statement: "*There is no membership fee*. It is expected that the necessary expenses of organization, distribution of literature, etc., will be paid from voluntary contributions."

Dr. Meltzer's noble address (p. 279) and his stirring appeal (p. 300), leave nothing to be said that would add materially to the reasons why all who may be engaged in the practise of medicine and in the advancement of medical sciences—*biochemistry among them*—should endorse the Brotherhood movement and enroll as members.

Because the significance of the following statements in the official "Appeal," in behalf of the Brotherhood, may not be fully noted by the casual reader, we single them out for special attention here:

"It is obvious that such a Brotherhood could not exercise an important influence at once. But our modest expectation for prompt results should not prevent us from attempting *now* to take the first step in the *right direction*. Many important results have often had small beginnings."

"It should be expressly understood that it is not the object of the proposed Brotherhood to influence the feelings and views of anyone regarding the problems involved in the present war."

Perhaps the most effective comment we can add to the foregoing is the following, from *Lancet*, on the "link of medicine":

We announce in another column the arrival in England from the United States of a complete medico-military unit, known as the "Chicago Unit," comprising the full medical and nursing organization for a general hospital of 1,040 beds. The establishment consists of 32 medical men (physicians, surgeons, specialists, a radiographer and a pathologist) and a nursing staff of 75 women, including the matron. The unit has been recognized by the War Office. Every one of our readers will admit with gratitude the practical sympathy of a splendid sort which is thus displayed by the United States, while we understand that similar units may be expected to arrive from America, taking their departure from other great cities, and animated with the same quick and deep desire to minimize as far as possible the horrors of war. From a private communication which we have received from one of the staff of the Chicago Unit, it is easy to guess that there will be no dearth of applicants in other American centers for what will necessarily be very hard and perhaps dangerous work. The medical men and nurses of the Chicago Unit were selected from several hundreds of applicants, every man's post could have been filled at least six or seven times, and a brisk competition prevailed among candidates for the nursing staff. The same rivalry will hold good elsewhere. And here we may add that a generous citizen of Chicago has personally offered to meet the large difference in pay between the salaries of military nurses in the British army and the salaries of nurses in the United States, where,

as is well known, skilled assistance of every kind is rated at a higher figure than prevails with us in this country. We are certain that the American nurses did not need money as an incentive, but it is equally certain that some of the most experienced of them could not have undertaken to volunteer unless the pecuniary footing of their employment had been made sure.

Medicine is here proving itself a real link between nations. When we get down to the simple fact of a man in pain, in sickness, and perchance in peril of his life, the differences that exist between one country or state and another, arising out of political conventions, trading regulations, unhealed quarrels or injudicious speeches, may, we see, *disappear altogether*, and the action of the United States *displays this medical spirit splendidly*. We must not forget that the war is practically a world war, and that its results now give rise to very serious mental and material trouble to the United States, the only first-class power still remaining neutral. While the greater part of the inhabitants of the United States, though maintaining political neutrality, are known to see eye to eye with the Allies in the rights of the quarrel, there is still a section among them who hold a different view, and who make the satisfaction that comes from complete unanimity very hard to obtain. Again, the trade of America is embarrassed and her financial position is complicated in many directions by a struggle in whose origin she at any rate had no part. Because of all this the European war is a subject of painful anxiety on the part of the inhabitants of the United States, and their action in helping our armies in the field by providing hospitals in accordance with the recognized military pattern, to be placed under the control of the War Office, is one for which we must be very grateful. Nor must we forget to express appreciation of the fact that among American surgeons and physicians who have come to the help of our sick and wounded are some who have left lucrative practices and important positions on hospital staffs, or in connection with universities, in order to lend their aid in the hour of need. This alone would show that *the medical impulse is at the bottom of their action*; the citizens of the United States are not taking sides so much with the Allies against the German as with the sufferers against the triple alliance of disease, privation and injury. *Lancet*, London, July 3, 1915.

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The BIOCHEMICAL BULLETIN is a monthly biochemical review. It publishes results of original investigations in biological chemistry, preliminary reports of researches, addresses, lectures, criticism, reviews, abstracts of papers, practical suggestions, biographical notes, historical summaries, bibliographies, quotations, questions, news items, proceedings of societies, personalia, views on current events in chemical biology, descriptions of new substances, methods and apparatus—any and all suitable items of personal and professional interest to students, investigators and practitioners of biochemistry.

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