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INTRODUCTION

The BIOCHEMICAL BULLETIN is a quarterly biochemical review. It publishes results of original investigations in biological chemistry and presents miscellaneous items of personal and professional interest to chemical biologists.

Biological chemists everywhere are cordially invited to forward contributions of any character whatever that will increase the value and add to the interest of the BULLETIN. Original contributions to research, preliminary reports of investigations, abstracts of papers, addresses, reviews, descriptions of new methods and apparatus, practical suggestions to teachers, biographical notes, historical summaries, bibliographies, quotations, news items, personalia, views on current events in chemical biology, etc., are solicited.

Editorial comment on the purpose, scope, reception and future of the BIOCHEMICAL BULLETIN will be found on pages 151, 363-364 and 576-579 of this volume.

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Biochemical Bulletin

Edited, for the Columbia University Biochemical Association, by the

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The BIOCHEMICAL BULLETIN is a quarterly journal of biochemical notes and news. It publishes results of original investigations in chemical biology and presents miscellaneous items of personal and professional interest to biological chemists.

Biological chemists everywhere are cordially invited to forward contributions of any character whatever that will increase the value and add to the interest of the BULLETIN. Original contributions to research, preliminary reports of investigations, abstracts of papers, addresses, reviews, descriptions of new methods and apparatus, practical suggestions to teachers, biographical notes, historical summaries, bibliographies, quotations, news items, personalia, and views on current events in chemical biology, are especially solicited.

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ANNOUNCEMENTS

American Chemical Society. Section of Biological Chemistry

The appended notice is a copy of a circular letter which has been forwarded to various investigators. We heartily commend the purposes and plans there stated to the favorable consideration of biological chemists.

"As a result of the recent meetings of the Biological Section of the American Chemical Society, the question of the organization of the Section into a Division is to be decided by the Council of the Society at its meeting in December in Washington. If the action is favorable, the Division will presumably be organized at that meeting. Your active interest in the Society and in the Biological Section at its December meeting is earnestly invited. In number of chemists present the meeting promises to surpass all previous meetings. On Friday afternoon, December 29, the American Society of Biological Chemists will hold a joint session with the Biological Section of the American Chemical Society.

"Papers for presentation before this section are solicited. Titles of such papers should be sent as early as may be to the Chairman or Secretary of the Biological Section, or to the Secretary of the Chemical Society."

CARL L. ALSBERG, Chairman,
Bureau of Plant Industry,
Washington, D. C.

ISAAC KING PHELPS, Secretary,
Bureau of Chemistry,
Washington, D. C.

ANNOUNCEMENTS (continued)

Chemists' Club of New York. Committee on Employment

The Committee on Employment of the Chemists' Club, 108 W. 55th St., New York, has now on file a number of applications from chemists seeking employment in laboratories and works. Employers wishing the services of such men should communicate with the Chairman, Prof. Herbert R. Moody, College of the City of New York, Convent Ave. and 140th St. Unemployed chemists should file cards, which may be had upon application to the Chairman.

The Committee extends its aid to unemployed chemists the country over and hopes that employers will use this means to get into communication with good men. No fees are charged.

The Columbia University Biochemical Association will cooperate with the Chemists' Club whenever opportunity to do so arises.

Hoffman and Kropff Chemical Co.

The Hoffman and Kropff Chemical Co., with office and factory at 619 Kent Ave., Brooklyn, N. Y., is now in a position to manufacture all the best grades of chemicals used in University laboratories. Dr. Alfred H. Kropff, Secretary and Treasurer of the firm, is a member of the Columbia University Biochemical Association. We cordially testify to his ability to satisfy the desires of biological chemists in need of fine chemicals and special preparations for research.

Biochemical Bulletin. Papers for the December Issue

ERNEST D. CLARK. A study of Lintner soluble starch.

CHARLES A. DOREMUS. A retrospect in biochemistry.

WILLIAM J. GIES, JACOB ROSENBLOOM, WILLIAM WEINBERGER, REUBEN OTTENBERG AND HERMAN O. MOSENTHAL. Abstracts of papers in a symposium on edema.

ROSS A. GORTNER. Melanins.

E. NEWTON HARVEY. The permeability of cells for dyes and alkalies.

LOUIS HUSSAKOF AND WILLIAM H. WELKER. Chemical notes on the egg cases of two species of sharks.

MAX KAHN. On the absorption and distribution of aluminium from aluminized food.

JOHN L. KANTOR AND WILLIAM J. GIES. Suggestions to teachers of biochemistry. 2. Methods of applying the biuret test.

ANTON R. ROSE. A cage designed for use in metabolism experiments on goats.

JACOB ROSENBLOOM. A review of the history of Bence Jones protein and multiple myeloma.

A. FRANKLIN SHULL. The effect of the chemical composition of the medium on the life cycle of *Hydatina senta*.



Ellen H. Richards

BIOCHEMICAL BULLETIN

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VOLUME I

SEPTEMBER, 1911

No. 1

MRS. ELLEN H. RICHARDS

MUCH has been said of Mrs. Ellen H. Richards and her pioneer work in Home Economics. I welcome the invitation and the opportunity to express in part the gratitude we owe her on the side of science. A journal devoted to the science of Biological Chemistry is a particularly fitting place for an expression of such appreciation, for, although Mrs. Richards called herself a sanitary chemist, and although she belonged to that department in the Institute of Technology, the vital import of her work and the direction of her energies included the application of chemical science to a more complete understanding of life. She has shown to the scientific world the inestimable value of a thoroughly trained, efficient woman, who, with a woman's instinct to understand practical situations, had the vigorous mental power which enabled her to put her ideals into realities.

Mrs. Richards was born in Dunstable, Massachusetts, in 1842. In 1875 she married Professor Robert H. Richards. She died at her home in Jamaica Plains, on March 30, 1911.

Mrs. Richards graduated from Vassar College in 1870 and three years later received the degree of M.A. from that college and of B.S. from the Massachusetts Institute of Technology. In the same year she was appointed instructor in sanitary chemistry in the Woman's Laboratory of the Institute, and later was given the chair of Sanitary Chemistry. This she held until her death.

Mrs. Richards achieved an eminence in science that no other woman of America has attained. Her thoroughness and vigor of mind were unexcelled by any man with whom she worked. She was rugged, alert, direct. Her mind possessed to an extraordinary degree the ability to separate the essential from the unimportant.

She had the constructive power of the engineer and a remarkable ability to *achieve*. She was a leader of women and of men. An authority of the highest order, she was placed on committees and in consultation with the foremost men in the country on questions of sanitation.

Mrs. Richards possessed the rare gift of vision. Her mind penetrated into conditions, enabling her to understand and foresee situations apparently closed to others having the same opportunity. Twenty-five years ago she foresaw the parts that sanitary science and preventive medicine were to play in the work of our educational institutions,—dreams which only the past few years have converted into actualities. It was this same prophetic vision which made her realize the urgent need of scientific and sanitary training for women in order that they might be able effectively and intelligently to carry on their work.

This training which she believed to be a necessity she chose to call Domestic Engineering, and it may be said that Mrs. Richards in demanding that women should have an intelligent understanding of the laws which governed their lives, has done more for their so called "emancipation" than any movement originated for that avowed purpose. She realized that the era for thoughtful, comprehensive work by women was at hand, and she stimulated and encouraged them in their efforts to this end. Speaking to a body of women, she once said: "We are trying to adapt ourselves to changed conditions. Do not let any one frighten or browbeat you out of that position." In originating, furthering, and leading the movement for Home Economics, Mrs. Richards sounded a call that will be heard around the world.

Her plea to all workers, men and women alike, was for *efficiency*, and the care, thoroughness and effectiveness of her own labors were an example of her teaching. It was the quality in Mrs. Richards which won for her the place of an undisputed authority. She believed it to be essential both for a proper grade of work and for the moral development of the individual. To use her own words: "The inner sense of ineffectiveness is the unrecognized cause of the restless discontent so prevalent to-day. No person who is accomplishing something, seeing it grow under his hands to what it was in thought, is discontented."

Mrs. Richards realized the tremendous waste of human efficiency due to ignorance of what constituted right living and overwork under wrong conditions, and she tried to show this and to present a remedy. She knew the individual must have the proper valuation of hygiene, pure water, proper food, and ventilation, and also a right understanding of house construction and decoration. She taught this in her lectures and in her writings. Health in the individual she believed to be the foundation for efficient work, and enthusiasm for health must be aroused if an effective life was to be maintained. In the preface to her last and perhaps her most important book "Conservation by Sanitation," Mrs. Richards writes: "The sanitary engineer has a treble duty for the next few years of sanitary awakening. Having the knowledge, he must be a *leader* in developing works and plants for state and municipal improvement, at the same time he is an *expert* in their employ. But he must be more; as a health officer he must be a *teacher* of the people to show them why all these things are to be. . . . Knowledge vital to the health of the people should be made as accessible as possible at as little expense and trouble to them as may be. There must be added the idea of making available this knowledge as quickly and completely as possible even if some of the application is premature."

Mrs. Richards was so insistent on sanitary conditions for fullest efficiency that she considered it much safer to err on the side of over-precaution than to neglect an opportunity of conserving health and energy. "It is better to believe that all dirt is dangerous rather than to hold it of no consequence how thick the dirt lies. . . . It is better to disinfect needlessly than to suffer longer the unchecked spread of disease. . . . It should not be counted against the sanitarian that he cried fire when there was only smoke and sometimes even only dust with no danger of fire. It caused a looking after danger spots, and was much better than the old medical practice which usually locked the stable door too late. Let the education of the people go on through mistakes, through excess of zeal, if it must—but go on all the time it must. As experience accumulates, wiser means will be found. Let the sanitary engineer seize his opportunity to lead in the application of all knowledge to the betterment of living conditions."

Most of Mrs. Richards' writings consisted of books and treatises on the application of chemistry to questions of sanitation. It is

interesting to know that although Mrs. Richards taught only men in her classes at the Institute of Technology, she believed that the greatest help for and reformation of our people in the matters of sane, intelligent living would be brought about at the hands of our women. Her faith in women was unbounded and that it was not misplaced is shown by the way thousands, all over the country, have responded to her call.

In the earlier years of her scientific career Mrs. Richards devoted her energy to the creation and development of her department of chemistry, but in her later years, her most earnest thought and her greatest interest were dedicated to the preservation and proper development of the home. She considered it the most fundamental basis for civilization and the most precious center for our development. Thus it became the focus of all her scientific efforts. And so it was that she believed that the greatest hope for the future development of the race rested upon such a scientific training of our women as would enable them to understand nutrition, hygiene, the proper care of children, and the orderly and sanitary conduct of the home and all its affairs. As she has often said: "The fundamental requirement for progress in applying science is the acquisition of science to apply."

It is undoubtedly true that a large part of Mrs. Richards' success as a leader and as one who achieved was due to her powerful personality. A woman of broad sympathies, she was wont to say: "Learn to look at people for what they can do, not for what they cannot." She had faith and a curious ability to bring out the best that a person could give. Her delightful humor and practical sense saved many a situation from failure. Her influence as a leader was subtle and indescribable. Plain, direct, honest,—with a passion for ethical truth, she gave one a sense of strength rarely encountered. She was intensely inspiring to both men and women, and deeply beloved. Professor Sedgwick has well said: "Other women may become experts in water analysis and preside over laboratories, but no one hereafter can possibly gain the peculiar historic equipment which fell to Mrs. Richards. Other women, may, and, no doubt, will make addresses and write books upon sanitation and homes, but no one else can ever do these things as Mrs. Richards did them, for the reason that she was herself an evolution, and represented an epoch."

EMILY C. SEAMAN.

THE MODERN BLOOD TESTS BEFORE TRANSFUSION*

REUBEN OTTENBERG AND D. J. KALISKI

(Pathological Laboratory of Mount Sinai Hospital, New York.)

Transfusion, when first introduced two centuries ago, and again when revived in the middle of the last century, had to be given up because of the occasional occurrence of certain unforeseen accidents. The better understanding of physiological principles led first to the abandonment of transfusion from lower animals into man, and then to the abandonment of indirect transfusion (injection of defibrinated blood).

The revival of direct transfusion in recent years is the immediate result of improvements in blood-vessel surgery. The success of modern transfusion, however, in the future, will probably depend largely on rather recently acquired knowledge of certain normal and abnormal qualities of the blood.

While it has been known ever since Landois' fundamental experiments that the blood of one species of animal is not indifferent to, but usually exerts a direct toxic action on, the blood of another species, it is only in recent years that we have learned that the blood is not always indifferent to the blood of other animals of the same species. Two kinds of phenomena may occur under certain circumstances, when such bloods are mixed: hemolysis and agglutination. The former, so far as we know at present, only occurs when one of these bloods is pathologically changed; *the latter occurs between many normal bloods.*

If blood toward which the patient's blood is hemolytic is used

* Experimental work related to this subject has been in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, under the auspices of the George Crocker Special Research Fund. See Ottenberg: *Journal of Experimental Medicine*, 1911, xiii, p. 425; Ottenberg and Friedman: *Ibid.*, p. 531; Kahn and Ottenberg: *Ibid.*, p. 536.

for a transfusion, the transfusion is followed by an intense hemoglobinuria with severe toxic symptoms and sometimes death. The results of mixing by transfusion, two bloods, one of which agglutinates the other, are not yet fully understood. Of the accidents reported in the literature, a number were probably (but in the absence of tests, not certainly) due to this cause. The authors have seen two transfusions of blood, which was agglutinable by the patient's serum, followed by death in each case in a few hours. On the other hand, they have seen a case in which the donor's serum was agglutinative to the patient's red cells, and in which nothing untoward happened. These discrepancies, Ottenberg has shown, are probably due to the fact that the amount of iso-agglutinin in a given volume of blood-serum is only sufficient to agglutinate a relatively small volume of cells. When a comparatively small volume of agglutinable blood-cells is transfused into a relatively large volume of agglutinative plasma (and the amount actually transfused is always small compared to the total blood volume of the patient), then agglutination may occur. But when the reverse is done, and a relatively small volume of agglutinative plasma is mixed with a comparative excess of red cells (and even in a very anemic patient the proportion is generally excessive), then the agglutinin is diluted and distributes itself without producing noteworthy clumps of cells. Furthermore, Ottenberg has shown that when agglutinable cells are transfused, there is active phagocytosis of red cells within the circulating blood of the patient. This renders it probable that even when no serious accidents occur, the body does not long retain such transfused blood.

These facts render it necessary to make agglutination and hemolysis tests before all transfusions, and to reject donors whose blood, when mixed with that of the patient, produces either agglutination or hemolysis. During the past two years, we have performed hundreds of such tests for over fifty human transfusions. A report on this work with details of the technic will be published at an early date.

THE TANNIN-COLLOID COMPLEXES IN THE FRUIT OF THE PERSIMMON, DIOSPYROS¹

(WITH PLATES I-3)

FRANCIS ERNEST LLOYD

(*From the Botanical Laboratory of the Alabama Polytechnic Institute and Agricultural Experiment Station, Auburn, Ala.*)

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I. INTRODUCTION

In order to explain the insolubility of tannin in the ripe persimmon fruit, I have, in a previous paper (Lloyd, 1911), presented evidence in support of the view there formulated, that, during the period of ripening, the tannin becomes associated with a second colloid, with which it forms an insoluble compound. The fact that "insoluble"² tannin occurs in the ripe and non-astringent fruit of the date and of the persimmon has been recognized by Bigelow, Gore and Howard (1906), by Vinson (1907) and by myself (1910),

¹ Presented, by invitation, before the staff and students of the Department of Botany, Johns Hopkins University, February 12, 1911.

² It is possible that this adjective is properly applicable, as here used, to the tannin only in the overripe fruit since, in addition to its fixation by combination with the second colloid, it becomes red (on exposure to air) and, probably, entirely insoluble and tasteless. I may therefore have erred in stating, in my previous paper (pp. 8 and 9), that the tannin in the ripe tannin-mass is "completely" insoluble in water or alcohol since, at this time, it is without color.

contrary to the previously held view that it disappears³ during ripening, by oxidation (Gerber, Aso, Sawamura), or is changed in one way or another.⁴ That this insoluble tannin presents certain physical peculiarities not to be expected in tannin itself has been noticed in the date by Vinson (1910), who speaks of the tannin-masses as fracturing "like grains of gelatin," and in the persimmon by Howard (1906), who describes at length the peculiarities of the physical behavior of the tannin-mass during the course of ripening. His observations have, in large measure, been verified by me, and I have so reported in the *Plant World*, as above cited. I found myself, however, in disagreement with this author on the score of the structure of the tannin-idioplast as a whole, and as to the origin of certain material, spoken of by him as "whitish particles" which "on further examination are found to be the expelled cell-contents, which on coming in contact with abundance of water . . . assume a white granular appearance" (Howard, 1906, p. 573). Concerning this material I have ventured the assertion that its granular appearance is not due to the assumption of this by the "cell-contents," and in differing with Howard, I should say clearly that I took his expression "expelled cell-contents" to mean the extruding tannin-mass. I think this is the normal and proper interpretation of the words in the connection in which they occur, but I mention the basis of my interpretation in order to avoid possible injustice. As to the presence, under certain conditions, of the "whitish particles" which have a finely granular appearance there can be no question, but at the time when my previous paper was written, I was unable to give a satisfactory account of their origin.

But although the curious appearance and behavior of the tannin-masses have been noted, there has been no attempt at their explanation although they are quite out of harmony with accepted criteria of tannin as such. Certainly, from the microchemical point of view, these are most interesting facts, and to the plant physiologist of very special moment. Using chemical methods, it has already been shown by Bigelow, Gore and Howard (1906) that, during

³Thompson and Bassett (1911) have advanced a stimulating view to the effect that tannin does not exist as such in the normal growing fruit (pear), but is rapidly formed, by an enzyme, on injury or removal from the tree.

⁴The various explanations which have been advanced, were reviewed by Dekker (1906) and by Bigelow, Gore and Howard (1906, p. 692).

the course of ripening, there is a decrease in tannin and a corresponding increase in marc. That the marc of ripe fruits contains tannin, and that this is insoluble, appears from their statement that it may be extracted from the marc only on warming with dilute mineral acid. In attempting a theoretical explanation of the insolubility of the tannin, these authors found themselves compelled to say that the tannin "either combines with some substance of low molecular weight . . . or is converted into an insoluble form by a change in the nature of the tannin itself." The former alternative they discarded as improbable, and adopted the latter, their position receiving support from Howard independently on microchemical grounds. I have already called in question the logical validity of this explanation. To say that the tannin becomes insoluble, it appears to me, is to state an unquestioned fact which needs explanation; to explain the change from solubility to insolubility by postulating a change in the nature of the tannin itself (which has not been shown to occur), does no more than to introduce the difficulty of explaining this change. Vinson (1910), treating of the date, believes that enzymic action intervenes. He bases his views on the inhibiting effect of heat on the change in question. Ripening with loss of astringency, he says, follows treatment such as to destroy protoplasm, but not enzymes; after temperatures sufficient to destroy the latter, astringency is not lost. Assuming the correctness of this conclusion, I would offer as an accessory view of tentative value that the enzyme invoked by Vinson has to do with the formation of a colloid with which the tannin may unite.

In its bearing on the present paper, it is important to note that, whatever the stage of ripening⁵ the fixation of tannin is not usually complete. Bigelow, Gore and Howard (1906) record the finding of traces of soluble tannin in all cases, at all stages of ripening studied by them. I, also, have found this to be true, but it will be understood, as indeed is the case, that the amount is not sufficient for detection as astringency, perhaps for quantitative reasons alone but probably, also, because of the nature of this free tannin. I have further found that at a stage when the fruit may still by courtesy be regarded as palatable, the amount of soluble tannin may

⁵ The term "ripening" is not restricted in this paper to the changes leading to edibility, but includes all later autolytic changes.

be considerably greater than it is still later. Mushiness and non-astringency are not constantly associated, as Howard (1906) has maintained; nor has the progress of the fixation of tannin reached its final goal, even with the last stage of edibility.

It is the purpose of the present paper to account, from the microtechnical point of view, for these phenomena; to show, namely, that the fixation of tannin during ripening is associated with the presence and, probably, accumulation of a second colloid (the view already advanced by me) and to discover the relation between soluble and insoluble tannin during the progress of ripening. To this end it will first be necessary to obtain a clear conception of the structure of the tannin idioplast, such as has not so far been had, either from Howard's account (1906) or from my own (1911). This physiological type of tannin-cell or idioplast is common to both the date and persimmon, and doubtless to many other structures including fruits (Tichomirow, 1904). But a prolonged study of them in preserved material of the date led me (1910) to no sure light regarding the structure and interrelations of parts in the living tannin idioplast, reliable as the methods employed by me may have been for determining the distribution of tannin in the tissues.

The present study is based upon fresh material of native and exotic species of persimmons, beginning with fully developed but still hard and wholly astringent fruit, and ending with specimens showing disintegration of the tissues. Fruits treated experimentally, with a view to differential ripening, have also been studied. While the details of method will appear as the account progresses, it will be well to say that the pressure of the cover-glass may be great enough to vitiate one's observation. In all cases, therefore, in which this disturbing condition might have obtruded itself, check observations were made without a cover-glass, and whenever single cells were under scrutiny, the cover was supported by a fragment of glass thick enough to prevent pressure or distortion of any kind.

II. THE NATURE OF THE TANNIN IDIOPLAST

An adequate idea of the tannin idioplast may be obtained only from the study of living material beginning with the hard unripe fruit. The cutting of a section results in the opening of many

tannin cells, and the spilling of their contents. The application of a tannin reagent then causes a general stain⁶ which obscures the structures of the cells it is sought to study, while the addition of water or glycerol causes disturbances which destroy their structure. There is only one way to see the tannin-cell without damage or distortion and that is to mount a small piece of tissue, cover with a light thin cover, and to examine the preparation where the air has been excluded by the fluids of the tissue itself. The addition of various agents will then enable the observer to test the nature of the structures seen. I particularly mention this, as the usual directions in text-books indicate the use of reagents and mounting media without reference to their physical effects upon the cell contents. It scarcely need be said that, for one studying such an object for the first time, it is necessary to identify the tannin cells in order to become sufficiently familiar with them to recognize them unstained.

In form, the cells in question vary from long, slender cylindrical to isodiametric (figs. 1, 2, 15), the general size and more prevalent form being more or less characteristic of the species (Howard, 1906) but also determined by the position of the cells within the fruit. The articulation with adjacent parenchyma cells is by means of facets and shallow pores; that with neighboring tannin cells by means of short cylindrical projections forming bridges (Howard, 1906). In an isolated tannin cell, these projections offer points of vantage for the observation of the behavior of the protoplasm and its inclusions under internal pressure to be described beyond. The wall reacts blue to iodine, indicating its pectocellulosic nature. During the early period of ripening, indicated by very slight softness in the fruit, the middle lamella is digested, and the tannin and pulp cells (as I shall hereafter call the non-tanniferous cells) are then easily separated, and can be more carefully studied. It is during this period that critical study of individual cells is possible for the first time in the course of ripening. Comparison with these cells in the still hard fruit shows that they may be had in the same physiological condition just after, as well as before, the initial softening of the middle lamella. In

⁶ Strasburger: Practical Botany, 6th ed., p. 429.

practice, I have found it possible to isolate a single cell, and the conclusions here recorded have been reached largely by the study of such.

The protoplast proper offers no visible peculiarities, but its relations and structure must be understood in order to comprehend the behavior of that inclusion, the "tannin-mass,"⁷ with which we are here especially concerned. The protoplasm which lies nearer the cell wall contains the spindle-shaped chromatophores (Lloyd, 1911, fig. 2) which are orange in color due presumably to carotin. The nucleus occupies a lateral position. In some places, more often at the end of the cell and in the projecting arms, one or more sap-vacuoles may occur, from which tannin is absent.⁸ This may be proved by the use of weak iron salts only with circumspection, since the color may, by showing through the vacuole, confuse the observation. Alkaloids may, however, be used with clear-cut results (fig. 8). Since both the nucleus and the vacuoles usually have dimensions greater than the thickness of the peripheral protoplasm, they cause this to project into the interior of the cell. The utricle itself occupies the vastly greater part of the volume of the cell, and contains one or more tannin-masses. The form of the body—assuming it to be single—may be understood if, in imagination, we should open the cell at one point and pour into the utricle a moulding material and thus obtain a cast of its interior. The cast would then represent the tannin-mass. In point of fact, this body is such a cast, and at this juncture I am emboldened to state my belief that the substance which composes it, aside from the tannin, is analogous to a cellulose hydrate (see Cross and Bevan, 1903, p. 26).

These peculiar cellulose compounds decompose spontaneously and, when in aqueous solution, form a jelly or coagulum which takes the form and volume of the containing vessel. This coagulum shrinks slowly, according to the authors mentioned, to 28.5 per cent. in 47 days, preserving, meanwhile, the form of the containing vessel. The application of heat causes rapid coagulation beginning at 70° C. As will appear, the substance of the tannin-

⁷ Howard used this term, and I use it in the same sense.

⁸ Went (1886, through af Klercker), and af Klercker (1888), showed that tanniferous and non-tanniferous vacuoles may exist side by side in the same cell.

mass coagulates and shrinks during ripening, and may be rapidly coagulated by heat, even before ripening has set in. From the purely histological point of view, this substance appears to belong to the group of pectic or mucilaginous substances found in the vacuoles of the mucilage cells of orchids, and probably of the parenchyma of some fruits, *e. g.*, of the pulp-cells in the persimmon itself. Before passing on to a critical examination of this peculiar "tannin-mass," we shall first consider its visible structure.

In my previous paper I described certain surface characters and showed that, in addition to lenticular depressions, there may be observed canals which extend from these depressions in various directions. A figure was presented showing these superficial appearances. Inasmuch as the description applied only to the surface of the tannin-mass, it failed to give any idea of the internal structure. I had overlooked the fact that the tannin-mass is penetrated by a complex system of canals, which are frequently paired (fig. 1) and are at many points expanded into variously shaped, but usually oval, cavities having the appearance of vacuoles (figs. 1-3).⁹ On first discovering these passages, they appeared to be due to the presence of protoplasmic strands, but the evidence that such is the case is too slight to warrant this view. It may be that during the earlier development of the cell some of the canals are so caused, but at this moment the point must be left for future study. At present I argue that they are not to be explained in this way, because of their peculiarities of form and direction, because they are often paired, and because of the number and shape of the cavities.

These canals and cavities are of great importance in enabling us to understand certain phenomena which otherwise are difficult to explain. They may be observed in tannin idioplasts in the tissue of a still quite hard fruit and must exist for a considerable period previous to this time. The figure (fig. 1) presented herewith shows the appearance of a tannin cell which lay in a position favorable for observation in a small section. The chromatophores and other protoplasmic structures, excepting the nucleus, are omitted in order to reduce the complexity of the drawing. This cell represents the

⁹ Tichomirow's remark, "Il y a aussi des inclusions ovoïdes ou sphériques de 30 microns à 65 microns" may refer to these vacuoles, but his very brief description does not enable me to decide (1904, p. 305).

average condition of complexity; many are far too intricate for graphic representation.

Attention may be drawn to a few, more important details. The canals are usually tubular; this is seen to be the case in their optical section. They are most frequently curved, sometimes sharply so, and are usually expanded into cavities which are spherical, oblate or prolate with reference to the canal as axis. Sometimes they are hemispherical or hemispheroidal as the case may be (fig. 4a). In number, these cavities may be few or so many along a single canal as to appear like a string of beads. A considerable number of canals may be, and normally are, confluent at some central point, to form a cavity with flattened arms extending in various directions. At its outer extremity a canal may end by opening into another, or out upon the surface of the tannin-mass (fig. 3). Here the mouth may be a minute pore no greater in transverse measurements than the canal itself, or it may be funnel-shaped, and either circular or a slit (fig. 2). It may open into a depression or immediately at the surface. The slit-shaped mouth, if it happens to be of that form, may extend so far around the tannin-mass as to appear as a sulcus (fig. 2), and this may be so deep as to cut half-way through the mass. Still again, just before reaching the surface, a canal may divaricate several times, and thus form a group of pores (fig. 3). Finally, a canal may become intricately branched in the interior of the mass so as to resemble the galleries of wood boring beetles, or so branch and intertwine as to produce an intricate web. These last named appearances have been seen only in definitively matured tannin-cells.

It requires only a slight realization of the condition which I have tried to describe briefly, to render doubtful the explanation which first occurred to me, namely, that these canals are caused by the bridging strands of protoplasm. The best explanation, I believe, is this, that the tannin-mass is composed of several, or a considerable number of, originally separate gelatinous bodies (probably contained in separate vacuoles), which have extensively but not entirely coalesced. The existence of separate tannin-masses, as occasionally observed, in separate vacuoles (distinguishable on contraction by glycerol) may be adduced in support of this view (fig. 5).

The form of the central cavity with its flattened arms is very similar to the branching arms of dough formed by the coalescing of bubbles of gas in rising bread, many of which are preserved in form by the baking, and are therefore seen on slicing the bread.

In order to explain, on the same grounds, the remaining details, especially those in definitively matured tannin-cells, we must invoke the behavior of gelatin and similar colloid masses during coagulation and shrinkage. Those who have used glycerol-jelly as a mounting medium to any considerable extent, are familiar with the curious tunnelling and cavity formation which frequently ensues upon the drying out of the preparation. The resemblance to these of the spheroidal and hemispheroidal cavities, and of the numerous blind canals which often occur, is close enough to warrant us in advancing this purely physical explanation of the appearances in the tannin-mass, especially if we may add that the soft and yielding elements of which it is composed may suffer displacements.

On this view, then, the canals are merely the spaces at the angles of intercepting planes of contact of adjoining masses of the jelly-like bodies which compose the tannin-mass, and are analogous therefore to the intercellular spaces between the walls of parenchyma cells. Their irregularity results from the irregularity of the component elements of the tannin-mass, aggravated by probable displacements. Shrinkage, or coagulation, after the fashion of cellulose hydrates and jellies as above indicated, will account for secondary changes leading to the formation of cavities of various shapes. If this shrinkage is not symmetrical, its lack of symmetry may be due to the uneven structure of the masses. For the purpose of the account which follows, I shall assume the correctness of this view, but while I believe in its essential truth, I do not overlook the possibility that further study of younger material than I have yet examined may make some modification of it necessary.

We may next ask concerning the contents of the canals and cavities within the tannin-mass. We can dispose of the originally superficial, usually hemispherical cavities mentioned by Howard (1906) and myself, as I have already done, since they are found

where a sap vacuole occupies the space. The cavity is therefore lined by protoplasm, this surrounding the vacuole (Lloyd, 1911, figs. 2, 8.) In every case, therefore, the canals which open on the surface of the tannin-mass, even though they do so on the hollow surface of a superficial cavity, *open into the thin space*, occupied by a film of water, *lying between the protoplasm and the surface of the tannin-mass*.

The canals are therefore such only secondarily, and mark the limits of former surfaces. They may have the width of a micron, seldom more, and frequently less, while the cavities may be several microns in diameter. One can see in them either nothing at all, which means of course that they contain water with no visible suspensions; or minute granular bodies, often large enough to be recognized as spherical, may be observed. I have found no method of differential staining to separate these contents visually from the tannin-mass, since whatever stains the latter obscures or occludes the former. The most natural expectation is that they contain tannin in solution in the water which fills them. This can be neither proved nor disproved by any means that I have used but that, under some conditions, they do contain a watery solution of tannin follows from certain observations to be cited beyond. There is some evidence also that the spherical granules consist of the same substances as those of the tannin-mass itself, inasmuch as similar masses giving a tannin reaction are sometimes found in a position rendering their reactions visible (fig. 34).

III. PHYSICAL AND CHEMICAL PECULIARITIES OF THE TANNIN-MASS DURING THE COURSE OF RIPENING

General considerations. While the fruit is still hard, the tannin-mass is so soft that it flows easily. After the process of softening has been fully initiated, the tannin-cells may be isolated successfully, without in the least injuring them, while the tannin-mass is still mobile enough to flow, like a weak solution of mucilage. If one selects a suitable cell having readily visible cavities, places a supported cover-glass over it, and, while under observation under fairly high magnification, runs water under the cover, the tannin-mass will quickly absorb enough water to cause the cells to burst

at one restricted point and flow out in a stream which mingles with the water (Howard, 1906). During absorption, one may observe that the cavities *become spherical, some may coalesce, and the canals between them are obliterated* (fig. 4a-c). If the fluid tannin-mass were of uniform structure and consistence, these cavities would flow in regular paths at a uniformly increasing speed toward the opening through which the fluid escapes. In point of fact they behave quite differently. They may be whirled about in eddies of constantly changing paths, here obstructed by an invisible something, and there moving with a rapid jerk. One of them, on reaching the opening in the cell wall, may be caught in a returning stream and find its way back into the interior. Ultimately they escape, appearing as vacuoles, carried forward into the surrounding medium by the flowing tannin-mass, and may be followed as I have followed them, for a half-hour, during which time they remain perfectly spherical. They finally fade away and become invisible. I have never seen one burst, nor have I been able by any means to produce any reaction within them. They appear to contain a few minute granules. I have not seen any definite limiting membrane. In a word, the fluid tannin-mass acts much as would a group of very much softened and swollen starch grains, or, better, of fluid droplets of jelly coalescing and enclosing bubbles of the water in which they were suspended.

Perhaps the most difficult part of my task has been to obtain a clear idea of the relation of this mass to the tannin it carries. As I have earlier shown (1910) and as is readily observable to be the case, the tannin-mass in the intact cell lies against the protoplasm. The film of water between them cannot by any stretch of the imagination be regarded as the container of the entire, large amount of free or soluble tannin which reagents show is present in the immature cell, nor would the case be helped materially by including the volumes of the canals and other cavities. The amount of soluble tannin is so great as to produce the well known astringency of the unripe fruit, and, when a weak reagent is applied instead of water, the bursting of the cell is followed by the rapid coloration of the escaping tannin and tannin-mass. I distinguish between them because the former, the free or soluble tannin,

appears as a colored granular precipitate, while the latter is a homogeneous mass of the same color, the color corresponding to the reagent used. Only one explanation of this has occurred to me, namely, that the tannin of the intact cell is all held within the confines of a colloidal compound which gives the form and body to the tannin-mass, but that upon swelling, followed by the bursting of the cell, so much tannin as is not firmly held chemically as a part of the colloidal compound, escapes by expression and diffusion into the surrounding fluid.

I wish to emphasize the importance of the observations on which this explanation is based. The bursting of a tannin-cell of a hard unripe fruit is followed by the outflow of a stream of colorless fluid, which mingles with the water. If, as the stream flows, a reagent, such as ferric acetate, be added, the stream may be differentiated into two parts, an outer sheath of precipitate and an inner core of mucilaginous matter (fig. 6c). The core may be observed to move forwards independently or, if the pressure of the cover be changed, backwards (fig. 6a-b). Both react by the proper change of color. Careful study will show that the precipitate (free tannin combined with the reagent and, possibly, with another substance) originates by *diffusion from the mucilaginous core*. The material of this core at length gives up no more free tannin, but itself still reacts, showing that it contains "insoluble tannin." It would seem therefore that the intact cell is filled with a colloid solution which contains a large proportion of water. The tannin is in part combined with the colloid and in part in solution in the water in the interstices of the colloid. If this be the case, one would expect that free tannin could be demonstrated within the cell, as, for example, by causing the tannin-mass to shrink. The difficulties of observation, however are great. It is impossible to get a preparation of a tannin-cell without the fruit-juice carrying the spilled contents of other tannin-cells. Washing or applying a watery reagent, causes bursting. With glycerol, combined with a very little ferric acetate, shrinkage occurs, but unequivocal evidence of the extrusion of free tannin has not been observed. It would rather seem that, so long as the tannin-mass shrinks, the free tannin is held imprisoned, as above described.

But though the extrusion of free tannin has not been observed by me within the cell-wall in the intact cell, it is probable that the behavior of the tannin-mass to alkaloids as reagents has a bearing on the problem. These reagents (caffein and antipyrin) have been employed with conspicuous success by van Wisselingh (1910) in his studies of *Spirogyra*. The reaction to these substances with the living *Spirogyra* cell consists in the formation of spherical globules, of viscid fluid, which coalesce to form larger ones and which redissolve on removing the reagent by irrigation. On applying either of the reagents to a tannin-cell, the bursting is followed by the extrusion of the tannin-mass. The coarse precipitate which ensues is visually separable as loose granular matter free in the surrounding fluid and as granular matter held together by a mucilaginous mass. Later on in ripening, the amount of loose granular matter decreases until it may be difficult to observe it at all. The precipitation in the mucilaginous mass, now obviously the tannin-mass, takes place less readily, but clearly within the limpid coagulum which holds the granules together. Still later precipitation occurs only at a distance from the surface, leaving a homogeneous zone (fig. 8), and, finally, not at all. The argument, then, appears to shape itself in this way, that so long as free tannin occurs in the tannin-mass, a granular precipitate occurs within it on adding an alkaloid.¹⁰ Inasmuch as, within the tannin-mass, only color reactions are produced by iron salts, it is impossible to conclude, from the character of the reaction, the presence of free tannin there, and we therefore are compelled to infer its origin from the fact of its extrusion. The reaction of the tannin within the tannin-mass by precipitation, when alkaloids are used, in view of the integrity of the tannin-mass as a whole and, in its definitive form, of the absence of precipitation, seems to offer direct evidence of the presence of free tannin within it.

We may now see how these conclusions align themselves with the behavior of the tannin-mass during the progress of ripening. When this has proceeded till the fruit is soft enough to be readily dented, and while the tissue of the mesocarp remains an opaque

¹⁰ The mucilaginous substance of the tannin-mass appears to act as a protector of the tannin in the case of an alkaloid, and, so far as to prevent a flocculent precipitate, against iron salts.

yellow, the tannin-cells will still be found to burst when placed in water. The tannin-mass causes this by its swelling, but the capacity for imbibition is now not so great as before. It results that, as Howard has described, the tannin-mass breaks out at some point, usually laterally, sometimes at the end of the cell. This bursting may now be seen to be due solely to the imbibing of water by the tannin-mass (Lloyd, 1191), and not to the behavior of the remaining cell contents to water. This is clearly shown by the interaction of the tannin-mass and the vacuoles, resulting in the displacement and final bursting of these within the protoplasm or at any rate within the cell wall (fig. 22, *a* and *b*). The protoplasm is disrupted, and the nucleus displaced and compressed. The cell-wall is finally stretched and then broken open, a rounded hole being formed by pushing off a piece of wall of relatively large dimensions. Out of this hole a rounded extrusion projects from the tannin-mass, its size depending on its imbibition capacity (figs. 12-14). This during ripening becomes progressively less until it is not great enough to effect the bursting of the cell, though the wall may be stretched nearly to its limit of strength. Under some conditions within the fruit, not well defined in my own mind as yet, the tannin-mass may stay permanently in this condition, unless dried out by evaporation, or it may pass into a state in which the total imbibition capacity is very small, even when completely dry. When in this condition, the tannin-mass appears slightly angular, pitted with a few depressions, transversely or spirally corrugated and frequently lobate. One or all of these appearances may be absent, but at any rate the mass appears to be, as it is, shrunken. In the interior, the canals and cavities, already described, may sometimes remain, or more usually, they may be entirely absent. The number of superficial pits or lenticular cavities may be greater than before. This condition, thought by Howard to be constant, may result, I think, from the displacement of the internal cavities to the surface of the tannin-mass. An intermediate condition, in which the internal cavities are present, but from which the canals are absent, indicates this. But such a condition may arise only by internal compression exerted in all directions, and this, in turn only by the swelling of the tannin-mass sufficiently to exert pres-

sure on the inside of the cell-wall. Whenever the canals and cavities, therefore, are absent from the tannin-mass, when in its definitive condition, this must have undergone a period of swelling during ripening. It is of importance to record at this point that the tannin-masses in a fruit acted upon for three days by acetic acid vapor (Oct. 17, 1910) remained permanently in the swollen condition and, after three months (Jan. 25, 1911), still show no change. They behave in this way also in some fruits not treated, while in others of the same variety they passed into the shrunken condition. Shrunken and swelled tannin-masses occur in the same fruit. At this time the cells are dead, but the tannin-mass may swell on the addition of water, and the expression of free tannin, as described beyond, may take place, in a manner identical with that in a living cell.

The swelling, when it takes place, appears to occur at the time when the middle lamella is broken down by digestion. It is now that the fruit becomes mushy or even watery, the parenchyma cells being at best loosely attached, or literally floating in the fluid. Despite the high osmotic equivalent of this fluid (for it has the consistence of thick syrup), the tannin-masses absorb water, thus accounting for the swelling. That the tannin-masses, if they are capable of swelling at all, will do so in a normal solution of potassium nitrate, accords with this observation.

The fact that during ripening the tannin-mass gradually loses, up to a certain limit, the power of swelling is a remarkable fact. It may be explained, perhaps, by the progressive combination with tannin, just as we may imagine a body of cellulose becoming less capable of imbibition by an increase in the amount of fats, as occurs in the formation of a cuticle. But it remains to obtain experimental proof that this sort of a change can be brought about by tannin.¹¹ Here it may be said that the tannin-mass swells much more in ammonium hydroxid solution¹² (fig. 7) than in water, though finally in the definitive condition there is little or no observable increase in dimensions.

The behavior of the soluble tannin. Correlated with this

¹¹ Gelatin alone and gelatin-tannin offer analogous conditions.

¹² Weak ammonium hydroxide solution is, apparently, as effective as a strong solution.

change from a fluid condition to one of relative immobility, the amount of free, or "soluble" tannin decreases. The behavior of this under the special conditions of investigation, as well as in the fruit, will now be described. It will make for an easier understanding of the facts if it be made plain at once that there is always some free tannin, no matter how far advanced in ripening the fruit may be. Indeed, Bigelow, Gore and Howard (1906, table II) reported finding traces of tannin in all their analyses. This is not explained by them, and might very well be thought to be of no significance from a chemical point of view. From the present one, however, the fact is of importance. To show that free tannin is always present, I have examined freshly made filtrates of dozens of fruits, in all stages from incipient ripening to complete disintegration. In order to eliminate the possibility of tannin-extraction by fungi, I have kept fruits in chloroform vapor and with thymol. If a fruit be allowed to stand on a plate, it will in time pass into a condition when the skin breaks and the fluids ooze out, free from the cells. This fluid contains a readily demonstrable amount of tannin. As an extreme example, I find free tannin also in the fruit already mentioned which, after treatment for three days with acetic acid, has stood for three months. I think we may conclude that, aside from fruits which have been treated with substances which unite chemically with tannin, there is always a small amount of tannin which fails to become fixed and is therefore non-astringent. The amount may be too small to be detected through its action on mucous membranes.¹³

From its fluid condition in the hard fruit, the tannin-mass passes, always gradually of course, into one of semi-coagulation. When in this condition, upon the addition of water to the preparation, the cell bursts, the tannin-mass breaks out in irregular fashion (fig. 6c). Its behavior suggests that of the albumin of an egg after being cooked by the method which produces "cod-dling." Upon extrusion, a cloud of free tannin is seen to surround it so rapidly that one has the same difficulty in ascertaining its origin as when the tannin-mass is quite fluid. It is, however, clear to the observer that the tannin-mass retains whatever shape

¹³ I am indebted to Mr. H. P. Bassett for pointing out that this tannin may be of the kind (iron-green) which does not combine with albumin, pectose, etc.

it may take. Its flow is quite limited and jerky; its limit of capacity for imbibition is evidently quickly reached.

The tannin-mass stains blue with iron salts, but remains homogeneous, while the free tannin appears as a precipitate, especially in situations nearer the cell. With ammoniacal potassium ferricyanid solution,¹⁴ a rose-red color reaction is evidence of free tannin, while the tannin-mass gives a brownish reaction. Alkaloids produce in the tannin-mass, at first especially on its surface, a pronounced granulation. At the same time, they appear to have a coagulating effect upon the postulated second colloid, so that the superficial layers are hardened. This results in the repeated eruption of the softer, uncoagulated inner portions through the fractures of the outer. Upon the exposure of a new surface, fresh precipitation is at once seen. The rapidity and extent of coagulation depends upon the strength of the reagent. A 1 per cent. solution of caffein is too strong for careful and deliberate observation. In practice I have repeated observations with either gradually increased or diminished strengths, between 1 per cent. and 0.1 per cent. and even less. The penetration of these reagents into the cell permits the conclusion that, upon reaching the tannin-mass or a portion of it remaining undisturbed, the course of the reaction is the same as in the erupted portion. One finds no evidence that there is any free tannin beyond the surface of the tannin-mass, nor in the sap-vacuoles. The alkaloids are of superlative value in showing the latter to be true. I have been able to follow the course of the reagent through the protoplasm into a vacuole and into the contiguous tannin-mass, without observing a trace of precipitate in the vacuole (fig. 8). Proof that it has penetrated the vacuole is seen in the rate of penetration and in the circumstance that the protoplasm becomes clear and translucent. An additional proof that the free tannin remains within the confines of the tannin-mass

¹⁴ Allen, A. H., Chemistry applied to the detection of adulteration. Chemical News, 29: 169, 189. 17 Apr. 1874. I believe that this is the first time that this reagent has been used in microchemical work. It is very excellent for the demonstration of certain difficult points, because of the delicacy of the reaction (it detects one part of tannin in 10,000 parts of water according to Allen and Fletcher), because of the distinct, but transparent reactions of the tannin-mass, and because of the rapidity of action.

is to be had by following the entrance of ammoniacal potassium ferricyanid solution into an intact cell. Previously (Lloyd, 1911), I had thought the more rapid local reaction of the tannin-mass to iron salts in an unbroken cell, observed also by Howard, to be due to the more rapid penetration of the reagent through the shallow pores of the wall. This is true at certain stages of ripening. The cell wall, however, undergoes slow hydrolysis, and after this has proceeded for some time, the reagent finds entrance at numerous points, independently of the pores. The staining then begins at these points, and by diffusion extends radially away from them, thus producing circular areas of color. As these enlarge and become more numerous, they coalesce, and thus the whole surface becomes colored. During the progress of this staining, one may observe a circular area formed on the edge of a superficial lenticular pit, proving that the reagent has reached the fluid in this pit. This, however, remains free from precipitate, a result which would be impossible if there was any free tannin there (fig. 9).

Somewhat later, the extrusion of the tannin-mass is sufficient in extent to produce only a sub-spherical bubble-like protuberance, usually on the side of the tannin-cell. This condition is well shown by a photograph in Howard's paper of 1906 (fig. 6). At this time the state of the tannin-cell is particularly favorable for the observation of certain points. The amount of swelling may be increased very considerably by using ammoniacal potassium ferricyanid solution (fig. 7) while the transparent reactions facilitate observation. In such cells the disruption and expulsion of the protoplasm, nucleus, and the internal cavities and canals may be followed. It is important to point out that the expelled protoplasm must not be confused with the granular precipitate of free tannin, either within the cell or without. I have been able to show clearly that the protoplasm remains, except under special circumstances, entirely free from tannin during ripening (fig. 10). I must also draw attention to the preservation of the cavities and canals, which may be found in the extruding portion of the tannin-mass (fig. 29), and frequently opening on the surface (fig. 11).

Upon the addition of ammoniacal potassium ferricyanid solution, the superficial layer of the tannin-mass may rupture, exposing

a freshly torn surface (fig. 7). From this surface there arises at once a rose colored cloud. The surface of the mass itself reacts by browning and does not show any disintegration. Such cells, upon standing in water without the addition of a reagent form either over the whole surface or over a restricted portion of it, a white granular mass, seen by Howard (1906) and regarded by him as due to the disorganization of the cell contents (the tannin-mass) on contact with water. The granular mass is really a precipitation membrane (fig. 13), which, if undisturbed, continues to enlarge as long as there is any free tannin supplied from the tannin-mass, but is unaccompanied by any change in the tannin-mass itself, in the sense that squeezing the water out of a sponge leaves the sponge unchanged. Again, if undisturbed, the sub-spherical membrane may form a tube from one point, and this may elongate till it enwraps the whole cell. The appearance of this tube is so similar to the hypha of a *Mucor* or *Achlya* as to puzzle one exceedingly until the origin is observed. I have however watched such tubes grow, and their reactions to tannin reagents show them to be free tannin precipitated on extrusion. By the use of alcohol, it is possible to extract from the tannin-mass all the free tannin, so that, upon subsequent treatment as just described, no such membranes, nor any tannin reaction except within the tannin-mass itself, may be observed. This I did by digesting tannin cells (isolated and in groups which remain fixed to the slide because of the coagulation of the adhering pectose by the alcohol) in alcohol for twenty-four hours or longer, and then submitting them to the proper tests. Although the tannin-mass shrinks somewhat, it recovers and swells on exposure to water.

We now face the question of the occurrence of precipitation membranes which give, on applying a test, the tannin reaction. It must be stated clearly that this material cannot be detected within the unbroken cell, and, as it assumes a finely granular structure on the outside, it must be inferred that the tannin which was free within the tannin-mass has now united with some other substance. Alkaloids as reagents have in favorable preparations enabled me to see the formation of granules just beyond the limb of the projecting tannin-mass (fig. 14). I have watched the

membrane formed in a watery preparation grow after the manner of a copper sulfate-potassium ferrocyanid membrane, and assume remarkable dimensions, having the appearance of a veil over the protruding tannin-mass (fig. 13). Such membranes are formed with great readiness if the pulp of a soft, edible but not overripe persimmon be mixed with, say, an equal bulk of water. If such a mixture be allowed to remain a long time so that fermentation, following the entrance of organisms, may proceed, the tannin-masses and these granular membranes will remain unaffected. They are, indeed, imputrescible tannin-compounds. I have found them to be more resistant to dilute acids than the tannin-mass itself, still maintaining their integrity after the complete hydrolysis of the latter. It seems therefore that the whitish granular matter in question is formed by chemical union of the escaping soluble tannin with a substance without the tannin cell, probably the pectose derived by digestion of the middle lamella. In support of this view, I offer evidence beyond.

We now examine the tannin-cell when it has reached a condition which, on adding water, is characterized by swelling sufficiently to stretch the cell wall, and even to rupture it, usually without the extrusion of the tannin-mass itself. The kind of result following exposure to water depends on whether a single cell or a small group is flooded or whether a larger mass of cells is merely surrounded with water. In the former instance, the rapid swelling may cause a rupture in the wall, preceded by the earlier described internal compression of the protoplasm and its vacuoles. By the use of a very dilute ferric acetate solution, I was able to watch the explosion of such a cell, when there was expelled a puff of tannin, which immediately formed a delicate blue spherical cloud at some distance from the opening. There was no extrusion of the tannin-mass. In another case, such an extrusion was observed to occur after the initial expulsion of tannin. If only a small amount of water be added to the preparation, so that the tannin-cells are in effect surrounded by the solution of pectose, or if they are mounted in fruit juice alone, a different behavior ensues. The swelling of the tannin-mass may now take place gradually, and the free tannin escapes under pressure from within by one or more minute pores

in the cell wall. The escaping tannin is precipitated and forms membranes of a variety of forms; tubes of varying diameter, hemispheres, spheres, chalices and various combinations (figs. 15-20). Their growth may be watched for a half-hour or longer, until the free tannin is exhausted. Reagents produce tannin reactions in these membranes. They may be preserved for future examination by adding a little weak glycerol, and beautiful preparations (though easily destroyed) may be made by judicious staining with methyl blue (fig. 20). If a growing tube comes into contact with the surface of the slide or cover, it will spread out to form a wider, flat tube. The direction of growth may be controlled by causing a light current in the surrounding water (fig. 19), though the formation of a membrane will cease if too much water be added. Through these tubes there may be observed no movement of granules, though that there is a movement of tannin in solution is a certainty. There is thus afforded a demonstration that the free or soluble tannin escapes from the tannin cell as a solution.

These events appear to be entirely independent of the living condition of the protoplasm. I have produced them two months later by the proper treatment of the pulp of fruit killed with acetic acid. The precipitation tube figured grew at the rate of 15 microns in 11 minutes, measured by an eye-piece micrometer (fig. 21). These curious precipitation membranes are formed of tannin which would never in the ordinary course of events be rendered insoluble *within* the tannin cell. I have found evidence, however, that, during ripening, this tannin (the excess over that which the tannin-mass can take up), is expelled by the temporary swelling of the tannin-mass and is thus forced out into the intercellular space now occupied by pectose, and combines, more or less completely, with this. Macroscopically, this may be followed in pulp which has been placed in a glass vessel, when without any addition of water a white substance is seen to form. That this occurs also in the fruits which are left entirely undisturbed, I have shown by allowing them to overripen without being touched or removed in any way.¹⁵

In the course of some days, the tannin-mass swells sufficiently

¹⁵ The accidental intercellular tannin is not due to this (Lloyd, 1911).

to stretch the cell-wall beyond the breaking point so that, in some fruits at least, one may find tannin-cells broken out in the fashion already described, on the addition of water. Inasmuch as the swelling is, in the undisturbed fruit, a slow process, accompanied by digestion of the cell-wall which results in weakening it in numerous places, it more frequently happens that the tannin-mass breaks out at as many points (fig. 24). It is evident that, during a certain phase of change in the already overripe fruit, the tannin-masses, as previously said on page 21 absorb water from the pectose solution resulting from the digestion of the middle lamella. The consequent swelling forces out the free tannin, and when pulp at this time is examined, the evidence of this is seen in the complete membranes or in fragments of them, still attached to (fig. 25), or broken away from, the tannin-cells. The same process may be observed under the high power, and every detail of the growth and change of shape followed, in preparations made by covering a droplet of the pulp and adding a little water or fruit juice. In the course of a half-hour a great variety of precipitation membranes will be formed.¹⁶

There is thus afforded direct evidence that, during this phase of ripening,¹⁷ some of the soluble tannin combines with substances outside of the cells in which it was secreted; Bigelow, Gore and Howard (1906, p. 700) rejected this view, but the reader will recall that I have already pointed out that they found traces of tannin in their extracts of quite ripe fruit. I venture the suggestion that a part of the "insoluble tannin" in the marc was the tannin which had escaped as soluble tannin and is therefore to be distinguished quantitatively from the strictly "insoluble tannin" combined with a second intracellular colloid. I have shown, furthermore, that this granular matter is in such a combination with tannin that neither alcohol, water nor dilute nitric acid destroys it, nor is the tannin extracted by water or by alcohol.

The extracellular tannin-colloid complex. The following ex-

¹⁶ One recalls the beautiful experiments of Le Duc (1910), the results of which, in many instances, present features in common with these here described.

¹⁷ At the time of edibility, when the amount of free tannin is for the first time not sufficient to produce astringency, the fruit has not passed into the condition when the tannin extrudes as a result of the spontaneous swelling of the tannin-masses, if indeed this always occurs.

perimental evidence is adduced in support of the view that the occurrence, on the outside of the cell, of granular matter giving tannin reactions is due to union between dissolved and escaped tannin and pectose or a similar substance on the outside.

If a few fragments of commercial tannin be mounted for microscopic observation in some clear fruit juice, the tannin will, after solution, at once unite with some constituent of the juice to form a granular precipitate. In water it does not, of course, precipitate but dissolves. The precipitate may be shown to give tannin reactions. It is insoluble in water. If a solution of commercial tannin be added to a solution of fruit juice in a test-tube, a cloudy precipitate occurs.

A strong solution of commercial tannin was run into a short fragment of a very fine capillary glass tube. This was mounted for microscopic observation in fruit juice with or without the addition of a weak ferric acetate solution. At the mouth of the tube a dendritic precipitate was formed. If not already stained, as it was in the ferric acetate solution of fruit juice, it gave the tannin reaction upon applying appropriate reagents.

While these experiments prove that a granular precipitate is formed in the union of tannin and some constituent of fruit juice, the variety of membranes obtained by the exudation of free tannin from the cells was not duplicated. This is, I think, because in these experiments, the tannin merely diffuses, while a mass movement is necessary for the production of membranes such as have been described. I have tried to imitate this condition, but the operation is so delicate that thus far I have been defeated.

The insoluble tannin within the tannin-mass. The question of the precise position within the cell of this soluble tannin, which under the internal pressure of the tannin-mass exudes through minute openings of the cell wall, now confronts us. The reader is asked to recall that the tannin-mass lies with its surface juxtaposed to the protoplasm; that this contains vacuoles proper to it; and that no evidence may be obtained that tannin dissolved in sap exists between the tannin-mass and the protoplasm. On the other hand, it may be shown conclusively that there is no tannin of any kind either in the protoplasm itself or in its vacuoles. Any

swelling of the tannin-mass results in a closer crowding of the protoplasm, in its disruption and in that of its vacuoles and in the concomitant stretching of the cell-wall. It is obvious, of course, that, no matter how close the tannin-mass crowds the protoplasm, there must be a film of water between them; indeed this must represent also, at least in part, the watery contents of the broken vacuoles. As a matter of observation, one may see the vacuole burst the tonoplast and spread out within the protoplasm itself. But, if we cause such a swelled tannin-mass to shrink, we cannot find evidence of free tannin in the space thus formed between the tannin-mass and the protoplasm. The natural conclusion appears to be that the tannin-mass gives out free tannin only upon swelling, as if the particles of free tannin were entrapped by the meshes of the associated colloid when these, under pressure, are contracted; and escaped when, the pressure being released, there is room enough to move.

There is at least one condition, however, which seems to negative the view that the free tannin may not be found just outside the tannin-mass. I have found that the tannin-masses in the fruit, already referred to (when exposed to acetic acid for three days and thereby killed), are found, after a lapse of three months, to swell and burst the cell wall in water. They have, however, passed into a condition of rigidity greater than that usually found in cells which burst, since the rupturing of the cell results from the *tearing-open* of the tannin-mass (fig. 27), due to the circumstance, apparently, that the inner part absorbs water more rapidly than the outer. Now, if there were free tannin distributed throughout the tannin-mass, we should expect to find it diffusing from a part or all of the freshly exposed surface (*f.s.*, fig. 27), as indeed happens when such is the case. The fact is, however, that free tannin escapes only from the edges of the fracture (*g.*, fig. 27), and, as I have repeatedly assured myself by close observation, from the fissure between the torn edge of the cell-wall and the tannin-mass. If a mass of such cells be mounted, so that they may remain clothed by the pectose adhering to them, and water added, the free tannin may be expelled slowly and may form precipitation membranes (fig. 21). If an unbroken cell be tested for tannin, however, the

occurrence of free tannin outside the tannin-mass cannot be demonstrated. We may, therefore, conclude only that the space between the cell wall and the tannin-mass (the protoplasm has largely disappeared in the case we are considering) is the avenue of escape of free tannin from the latter, and not a reservoir. This explanation, which is not entirely free from points of attack in view of the behavior of the torn cells just described, receives support from the behavior many times noted, that there is a greater extrusion of free tannin from the edge of the opening through which the tannin-mass has been projected, than from the tannin-mass itself (fig. 12). Between the two cases, however, there is a difference which readily catches the eye, but which it is difficult to describe or to illustrate by a drawing. From the torn edge of the tannin-mass from the acetic acid fruit, the escaping tannin appears to be partly composed of fine granules already formed by precipitation inside of the cell wall, and to escape in part in this form; in the other case there is no doubt that it escapes as a solution. The formation of precipitation membranes in the form of tubes from cells from the acid-treated tissue, however, appears to deny the correctness of this explanation. If, again, the free tannin existed as such between the tannin-mass and the cell wall, this itself would take up some of it and show the tannin reaction, and, though I have observed this to happen in hard fruits heated in a sugar solution, it was not the case here.

But if the evidence indicates that there is no free tannin in the space outside of the tannin-mass, there is no lack of it to show that there is free tannin on the inside. If the tannin escapes through the cell wall, it is impossible to say whether the origin is general or local with respect to the tannin-mass. If, however, the tannin-mass becomes exposed by bursting out through the cell wall, the distinction is evident. Thus, for example, the white, granular membrane, which forms a short time after the extrusion of the tannin-mass, may completely envelop it (fig. 13); on the other hand, it may arise from one or several separate points of the exposed surface removed as far as possible from the cell wall (fig. 29). I think that it is proper to conclude that this is to be accounted for by the presence of the canals which I have shown may maintain

themselves while at the same time flowing out with the tannin-mass, and thus open out on the exposed surface.

I have seen somewhat more striking examples of the very local escape of free tannin from cells which have swelled considerably while still in the fruit, and on being placed on a slide with water added, have burst. One such cell is figured which had burst, tearing apart the two ends (fig. 28). From several different points on the exposed portion of the tannin-mass variously shaped but small precipitation membranes had formed.

Such instances as these can hardly be interpreted except by admitting that the escaping free tannin finds its way from the inside of the tannin-mass by following some relatively easy path, whatever this path may be. This conclusion flows somewhat more obviously from the following instance. I have chanced to observe a few cells in which there were two tannin-masses with their adjoining ends approximately lying in the equatorial plane of the cell. From these cells there was an escape of free tannin also in the equatorial plane (fig. 31). I have also noticed that there is a greater tendency for the precipitation membranes to be formed along the superficial furrows which are often present (figs. 32, 33). These examples must, I think, mean that the furrows between independent masses or contiguous lobes of the tannin-masses afford paths along which the free tannin travels more readily than elsewhere.

Assuming that the spontaneous swelling of the tannin-mass always occurs (though I admit that I have been able to show only that this is *frequently* the case), the tannin-mass may pass into the final condition which is best described by the adjective shrunk. I speak conditionally, since I have fruits in my laboratory at the moment of writing which have been standing in open and in closed vessels, protected with antiseptics and not, and in various stages of drying out, and I find that this final shrinkage is not by any means sure to occur. Mere exposure to rapid drying in the air causes shrinkage, but not in a symmetrical manner. By this time, when it does occur, the protoplasm is not only dead but much disintegrated. The form and structure of the tannin-mass in this condition I have already described above. In this regard only one

point of importance remains to be dealt with, namely, that in some fruits I have found that, at one end of the cell especially there appears an increased number of tannin-masses, in the form of spheroidal bodies of various sizes, down to granules (collected in irregular masses), the form of which must be inferred from the gradations in size observable (fig. 34). Sometimes this apparent additional matter appears at other points. I am unable to assert that there is an addition to the total amount of tannin-mass substance, but I am equally unable to say that there is not. I have never seen this condition in an unripe fruit, but certain appearances, such as the increased lobulation, suggest the possibility that the additional bodies may have arisen by constriction during shrinkage, or that the shrinkage of already separate portions makes them more readily observable. But it is more difficult to account for the finely granular matter in this way. In this final condition, alkaloidal tests are negative, since they produce no precipitation within the tannin-mass. This shrinks somewhat when acted upon by the reagent, becoming less transparent, slightly yellowish in color, but otherwise no result follows. Color reactions follow the use of metal salts. Inasmuch as there is every reason to believe that the tannin-mass is incapable of further spontaneous change, interest is now diverted to the question of the nature of this substance.

It has already been pointed out that in the very immature tannin cells, the tannin-mass occupies the whole of the vacuole in which it occurs, but it has also been made clear that, when such cells burst, as they do in water, the escape of the tannin-mass as a fluid is accompanied by a diffusion of free or soluble tannin into the surrounding water in which the preparation is mounted. It has been, however, always possible to see that the tannin-mass itself has its own integrity. While it mingles with the water, it has the appearance of a soft gelatinous mass. Whether the swelling is indefinite or restricted, I am not able to say, but my impression is that in this respect, it is analogous to swelling starch. Its behavior, however, may be due to its union with tannin. This is therefore a point for further investigation.

The relatively large amount of free tannin in such cells appears to be due to the relatively small amount of the associated colloid

in the tannin-mass. Within the limits of the size of the cell, however, this associated colloid always occupies the whole space; it takes the form of the "containing vessel." This is shown by material of both the date (*Phoenix*, Lloyd, 1910) and the persimmon, which has been preserved by means of copper acetate and with ethyl nitrite vapor. A still more illuminating piece of evidence has been obtained by dropping blocks of hard persimmon mesocarp into boiling 20 per cent. cane sugar solution, and allowing the boiling to continue for two hours. In such material the tannin-cells do not burst, but most, if not all, of the free tannin escapes and combines with the remaining tissues, so that, upon adding ferric chlorid to a section, the whole gives a strong tannin reaction. The tannin-mass, that is, the compound colloid in question, is now hard, and cuts, with the razor, like hard cheese or coagulated albumin, showing furrows and fractures of the expected kind. Alkaloids produce in it no precipitate. The material therefore contains no free tannin, is coagulated, and is seen to occupy the whole space of the vacuole. The absence of any internal structure, such as I have already described, indicates that there was some swelling, since swelling, by setting up internal pressures in all directions, is the only way by which the internal structure is eliminated.

If this view be correct, the change in the tannin cells during ripening consists in an increase in the amount of the colloid. Vinson's (1910) very interesting experiments on the effects of high temperatures on the fixation of tannin may therefore mean that if the protoplasm is killed, the additional secretion of the associated colloid is prevented. It is quite likely that an enzyme is one factor involved, as Vinson thinks he has proven to be the case. The killing of the protoplasm, releasing "intracellular enzymes," would, however, result in the softening of the fruit. This result would occur, I think, quite independently of the behavior of the tannin. In the Japanese procedure of artificial ripening, appreciable softening of the pulp must not occur, but the tannin must have become fixed. Though other possibilities are not excluded, such as the possible increase in the tannin itself during some stages of ripening, I think it probable that the successful processing of persimmons consists in an operation during which the increase of the associated

colloid, with which the tannin may unite, is made possible, while the other processes are largely suspended. Following Prinsen-Geerligs' work on the banana, Gore (1910) has shown that this may occur in an atmosphere of carbon dioxid or of pure hydrogen.¹⁸

This explanation harmonizes also with the facts obtained by Bigelow, Gore and Howard (1906), which show that, during ripening, the marc increases inversely as the tannin in *their extracts* decreased. There is, however, little or no decrease in the absolute amount of tannin in the fruit, for its combination with the associated colloid results in its conversion into a form which, being unextractable (or only very slightly so) by alcohol or water, remains in the marc. The marc itself also increases in bulk because of the physical condition of the associated colloid with which the tannin unites. In an unripe fruit, the associated colloid is extractable; in the ripe fruit it is not.

During ripening, the tannin-mass contains a decreasing amount of water. I have used the word dehydration (Lloyd, 1911) to describe this change, but I think that while graphic, it may not express the truth except in a relative sense. The reduced power of swelling may be due alone to an increase in the amount of colloid which, as fast as formed, unites with the free tannin, or because the complex thus formed has less capacity for imbibition. That there is an actual increase there seems to be no doubt, from the facts afforded by the study of abnormal tannin-cells, which, for some reason, have died *in situ*. Such cells become red or brown in color, and have been observed by Aso (through Howard, 1906) and by Howard (1906), but I believe, wrongly interpreted. On exposure to air, the tannin-cells which are normal take on the same red coloration. There is, therefore, nothing peculiar to these cells in the color. This is rather in the form of the tannin-mass, which in these dead cells, shows much *greater amount of shrinkage*, as is shown by the more extensive superficial pitting in those cells which have died in a quite immature condition of the fruit. This shrinkage is a measure of the amount of colloidal material, or, if one chooses to put it in another form, of the amount of water in it.

¹⁸ Communicated to me by Mr. Gore during a conversation with him.

The foregoing considerations lead us to the conclusion that the task remaining is to separate the tannin and the associated colloid, which, I have argued, combine with each other during ripening. The ease with which the ripe tannin-cells may be separated from the softened pulp, as Vinson (1907)¹⁹ found to be the case in the date, enables us to get quantities of the tannin-masses for chemical investigation. I have found (1911) that weak mineral acids cause the synchronous solution of the tannin and of the associated colloid, but I obtained a few cells which showed a remnant of the latter which did not give a tannin reaction. Since then I have found that, by using very strong nitric acid and by careful boiling for some minutes, the end-point being indicated by a swelling of the tannin-masses, the tannin may be completely extracted,²⁰ without attacking the associated colloid appreciably. This colloid is finally hydrolysed. Concentrated sulfuric acid gives a similar result, with some minor differences of no immediate moment.²¹

This experience seems to mean that it has been possible to split off the tannin before marked hydrolysis of the associated colloid occurs. And it is now possible to investigate this substance with a hope of throwing light on the true nature of the associated colloid. Some preliminary work indicates that it is a cellulose mucilage, a view supported by its behavior toward mineral acids. Such a substance, in superficial appearance, occurs also in the non-tanniferous pulp-cells; but whether this is identical with that in the tannin-cells remains to be seen. That in the pulp-cells is not miscible with water, contracts (becoming highly refringent) upon treatment with glycerol, and is broken up into globules by alcohol. Upon addition of water, the cells burst, as do the tannin-cells,

¹⁹ Vinson who speaks of the tannin-mass as insoluble tannin states that it does not give the tannin reaction unless the dates are fresh, "even after boiling with acids" (1907, p. 261). I have, however, found it quite easy to get the reaction even in market dates.

²⁰ Vinson apparently failed to see the significance of his observation, embodied in the previous footnote, that "after boiling with acids" tannin cells from the date fail to give a reaction.

²¹ For further details concerning the behavior of the associated colloid toward chemical reagents see Lloyd, F. E.: Ueber den Zusammenhang zwischen Gerbstoff und einem anderen Kolloid in reifenden Früchten, insbesondere Phoenix, Achras und Diospyros. *Zeitschrift für Chemie und Industrie der Kolloide*, 9: 65-73. 1911.

and the jelly-like mass extrudes, or migrates into the surrounding medium, either accompanied by the protoplasm or alone. It is probable that this substance contributes to the difficulty experienced by Bigelow, Gore and Howard in filtering watery extracts. Whatever facts may be brought to light by a study of this, and of the associated colloids, which I hold tentatively to be a cellulose mucilage, they will assuredly be of interest.

IV. SUMMARY AND CONCLUSIONS

A brief setting forth of the views concerning the fate of the tannin during the process of ripening in the date (*Phoenix*) and in the persimmon (*Diospyros*) shows that the explanations heretofore advanced are inadequate. The presence of the tannin in ripe fruit, and the absence of astringency have been the basis of the statement that the tannin is insoluble. The contention here made is, that insolubility of tannin as such is not the fact, but that it has in large part combined with an associated colloid to form an insoluble colloidal complex. The evidence for this has been sought in the visible behavior of the so-called tannin-mass, in its physical relation to soluble or free tannin in the same cell, and in its behavior toward chemical reagents.

The tannin-mass has been shown to have an internal structure, consisting of a system or complex of canals, spherical or subspherical spaces, and lacunae which have a definite existence. Their origin has not been shown, but a tentative explanation has been advanced. Their behavior during the maturation of the tannin-cell, and their form relations as a result of the swelling of the tannin-mass, have been described in detail. The complex in question is broken up, the cavities (having the appearance of vacuoles) often taking a superficial position on the tannin-mass, by internal pressure produced by the swelling of the tannin-mass restricted by the cell-wall. They have been shown not to contain free tannin under normal conditions, but it is probable that they afford paths along which soluble tannin moves under pressure.

The material called the tannin-mass is shown to be a tannin-colloid complex, the second named term of which appears to be a cellulose-mucilage or allied colloidal substance.

It is important and of very great interest here that the idea that tannin in the cell-sap may be associated with protein was advanced by Pfeffer (1886, through af Klerker, 1888), but was shown by af Klerker to be open to serious question if not entirely untenable. The latter however saw evidence in certain physical peculiarities of the "Gerbstoffblasen" that in the plant cell tannin occurs in two forms, namely as a solution and "als nightfluessige amorphe Massen oder Ballen" which, with increasing age of the cells are dissolved (aufgelöst) and disappear. His final summary statement "Eine durch Plasmolyse bewirkte Ausscheidung *fest-zweichen Gerbstoffes*²² kommt häufig in den Gerbstoffvacuolen vor" indicates that he did not analyze the situation any further, though it should be said that, while he sought for other materials (oils, inulin, albumin) without success, he spoke of the tannin-mass, as a "Gerbstoff einschliessende Körper."

The physical characters of the tannin-mass are those of the associated colloid, aside from those, such as its possible unlimited solubility or imbibition capacity, which may be influenced by the presence of tannin in the combination.

However fluid this combination may be in the unripe fruit, it has a definite structure and consistence which can not be ascribed to a tannin-water solution. In this condition, the tannin-cells burst in water, and the tannin-mass escapes. From it there is a synchronous escape of soluble tannin, which may appear as a finely granular precipitate. Heat suitably applied so as not to burst the tannin-cells, coagulates the tannin-mass, so that it takes on much the appearance of that in the mature tannin-cell.

During the course of ripening, the amount of free or soluble tannin is reduced. So long as any free tannin is present, it may escape from the tannin-mass in a manner described in detail. It is shown that this escape may also occur in the undisturbed fruit. The manner of escape is held to indicate its previous residence within the confines of the tannin-mass itself. The formation of white granular matter on its escape is shown to be due to its combination with a substance outside the tannin-cell, probably pectose. It is shown that the free tannin is not wholly fixed, but that there is

²² Italics are mine.

a small amount of tannin which does not unite with the colloids present.

The view is advanced that, during ripening, the supposed cellulose-mucilage increases in quantity and that sufficient is at last formed to engage most of the tannin. When mature, the tannin-mass contains nearly, but usually not entirely, all of the free tannin in a condition²³ which prevents its extraction by ordinary solvents and its detection by *alkaloids* as reagents. In order to separate all the tannin from the tannin-colloid compound, drastic chemical methods must be used. Decomposition of the tannin by means of strong mineral acids, carried on more rapidly than the hydrolysis of the associated colloid, has been used to effect this change. Some evidence has been advanced to indicate the nature of this associated colloid. It is probably a cellulose-mucilage.

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²³ Dr. J. Dekker suggests (*in literis*) that unless the tannin has been changed chemically it may still be partly extracted by alcohol or water. Such a change, indicated by slight reddening of the tannin mass, ensues slowly in the overripe fruit.

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VI. EXPLANATION OF PLATES

f.s., fractured surface of tannin-mass; *o.*, openings of canals on surface of tannin-mass; *v.*, vacuole; *tm.*, tannin-mass; *tg.*, free tannin.

FIG. 1. Tannin-mass; internal and superficial structure of canals and cavities. Unripe, firm fruit. Variety "*Taber.*"

FIG. 2. Tannin-mass from a soft, ripe fruit. "*Taber.*"

FIG. 3. Portion of tannin-mass, showing, especially, the way in which the canals open on the surface. *Diospyros virginiana*.

FIG. 4. *a.*, Portion of a canal with three adjoining cavities; *b.*, the same, showing partial effect of internal pressures; *c.*, final effect. *D. virginiana*.

FIG. 5. End of an idioplast: *a, b, c.*, separate tanning-masses shrunk by glycerol; *a', b', c'*, the same on adding water. "*Taber.*"

FIG. 6. *a.*, Stream issuing from idioplast of an unripe fruit; *b.*, tannin-mass flowing back, leaving free tannin behind; *c.*, tannin-mass issuing as a partially coagulated gel, and diffusing free tannin therefrom; *a, b, D. virginiana*; *c, Taber.*

FIG. 7. Effect of ammonia on the tannin-mass (compare with fig. 13).

FIG. 8. End of idioplast with two vacuoles, without tannin. Coarse granular alkaloidal reaction in, but not near the surface of, the tannin-mass.

FIG. 9. Reaction of tannin-mass at the edge of a superficial depression, which contains no free tannin.

FIG. 10. Portion of completely matured idioplast, the protoplasm dead, but showing no tannin reaction.

FIG. 11. Extruding tannin-mass with opening on the surface.

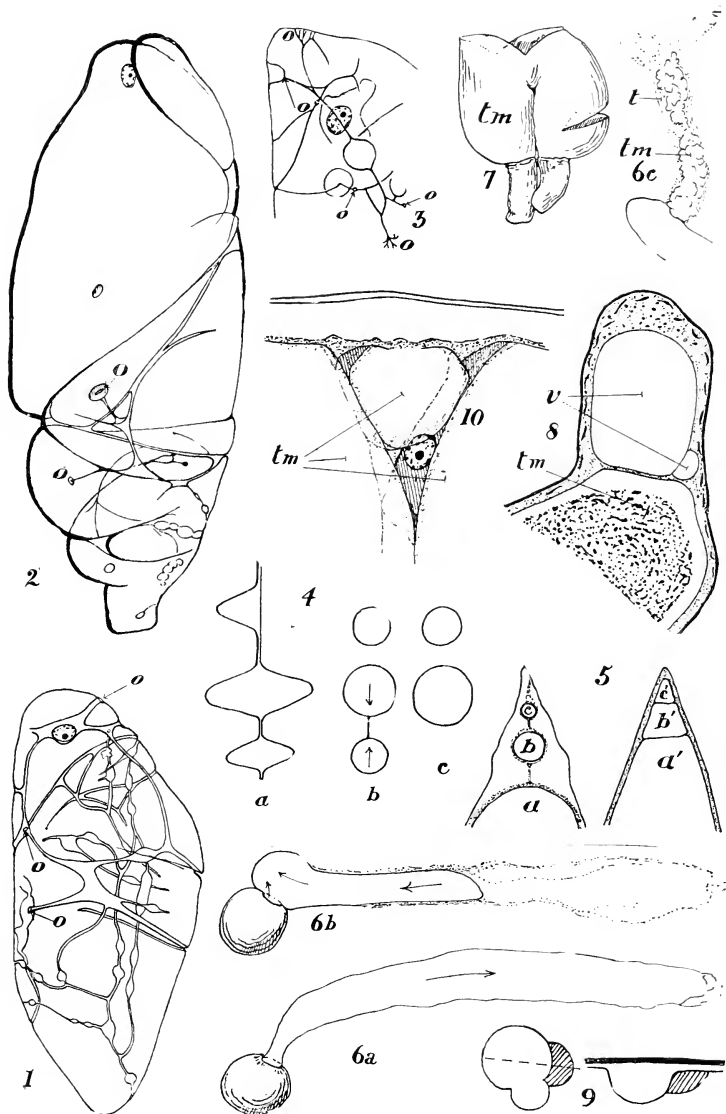
FIG. 12. Extruding tannin-mass, showing an apparently greater diffusion of tannin from the torn edge of the cell wall.

FIG. 13. Extruding tannin-mass, surrounded by a growing precipitation membrane.

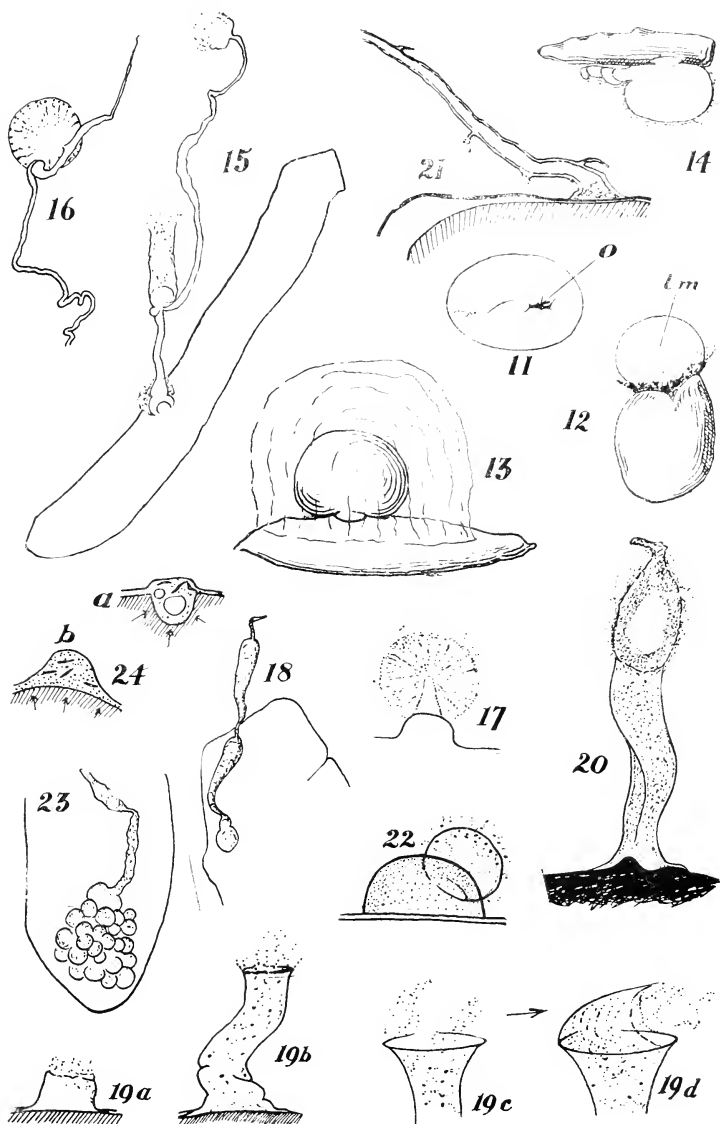
FIG. 14. Precipitation of free tannin just beyond the surface of extruding tannin-mass. 0.1 per cent. caffein.

FIGS. 15, 16, 17, 18. Various forms of precipitation-membranes and figures.

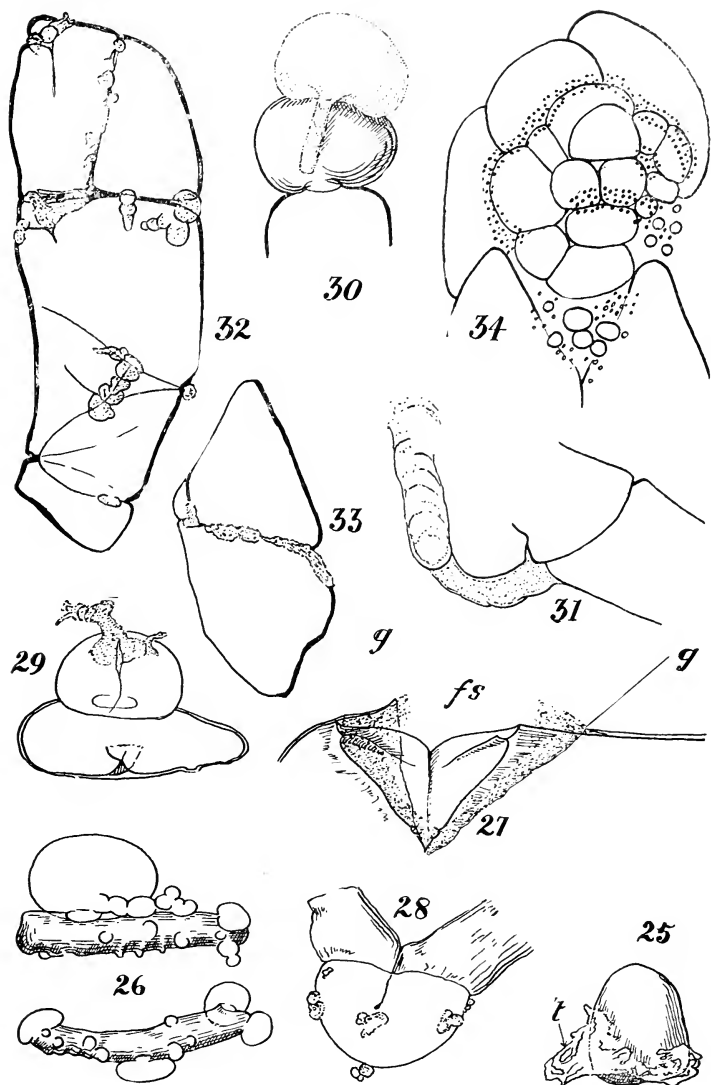
FIG. 19. *a-d.* Four stages of a growing tubular precipitation-membrane, the final shape of which (19, *d*) was produced by a current of water flowing in the direction indicated by the arrow.



LLOYD: TANNIN-COLLOID COMPLEXES.



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FIG. 20. Tubular membrane spreading out at its end on the cover glass. Stained with methyl blue.

FIG. 21. Ditto, formed from a cell of a fruit three months after treatment with acetic acid vapor.

FIGS. 22-23. Spherical and hemispherical membranes.

FIG. 24. *a*, Depression in tannin-mass filled with protoplasm with vacuoles and chromatophores; *b*, the same after partial swelling of the tannin-mass. Arrows show direction of pressures.

FIG. 25. Idioplast from ripe fruit, with adhering tannin membranes. *Taber*.

FIG. 26. Idioplasts, burst *in situ* in a fruit ripened at a low temperature.

FIG. 27. Same fruit as fig. 21. Tannin-mass fractured by imbibition, the diffusion of free tannin taking place from the torn edges of the cell wall.

FIGS. 28-30. Diffusion of free tannin forming membranes from openings in the exposed surface of extruding tannin-mass.

FIGS. 31-33. Membranes formed at the fissures in the tannin-mass.

FIG. 34. Tannin-masses at the end of an idioplast, showing independent bodies of all sizes.

ON THE RELATION OF BIOLOGICAL CHEMISTRY TO HOME ECONOMICS¹

WILLIAM J. GIES

Biological chemistry, considered from the standpoint of its greatest usefulness, is primarily chemical biology. Its foundations are physics, chemistry and biology. Biological chemistry, as a science, is chemical knowledge of biological substances and processes, *i. e.*, substances that commonly enter, or that are produced in, or are derived from, organisms; and processes that ordinarily affect, or that occur in, or are modified by, organisms. Biological chemistry, as an art, is fundamentally chemical in method and primarily biological in purpose. The biological chemist seeks to determine by chemical means the nature of biological phenomena; he does not attempt to establish purely chemical laws through biological agencies.

Among the leading subdivisions of biological chemistry are physiological chemistry, pathological chemistry, pharmacology, agricultural chemistry, botanical chemistry, and bacteriological chemistry. Physiological chemistry is the chemical branch of physiology, *i. e.*, the chemistry of substances and processes and conditions pertaining to healthy organisms. Physiological chemistry is physical chemistry, inorganic chemistry and organic chemistry applied to physiology.

The substances that engage the attention of the physiological chemist are very numerous in kind. They range in diversity of molecular characters from the very simplest inorganic salts like sodium chloride, to the most complex organic compounds like nucleoproteins. The processes that form a large portion of the subject-matter of physiological chemistry are not only numerous

¹ An abstract of the official report of an extemporaneous address, and the ensuing discussion, at the Boston Meeting of the American Home Economics Association. Reprinted, with the consent of the editor, from the *Journal of Home Economics* for December, 1910 (volume II, pp. 619-625).

in kind and varied in character, but often are also unique; and many are wholly inexplicable on the basis of our present knowledge.

Normal organisms are specially constituted to achieve two fundamental biological results, *viz.*, their self-preservation and reproduction. In effecting its own nutrition, an organism directly adds to its mass and enlarges its structure, as in growth; or it mainly "repairs" its parts, directly or indirectly from nutrient material, as in maturity. Nutrient materials (as a group) also supply energy, in one form or another, which is available for transformations to the advantage of the organism. Normal reproduction is, in effect, the nutrition of immature organisms derived directly from mature ones.

These two major functions of normal organisms (self-preservation and reproduction) are, in effect, the development, repair and reproduction of structure, as well as the maintenance of continuity in coördinated chemical processes. The mechanico-chemical functions of an organism (external respiration, heart-beat, excretion, etc.) are contributory to the highest efficiency of the major functions just mentioned, and, therefore, are essentially nutritional in ultimate significance. The chemical nature (composition) of an organism, as well as the peculiarities and functions of its tissues, are best understood when studied with due regard for the relations of all the parts and constituents to the nutritional processes.

Normal nutrition, whether in growth, in maturity, or in old age, is primarily chemical in method and constitutes the chief subject-matter of physiological chemistry. Pathological chemistry compromises the facts pertaining to nutrition in disease. The hygiene of nutrition is obviously a matter of fundamental personal and domestic importance.

With these ideas on the general nature and practical import of biological chemistry before us, I think I can best perform the function allotted to me by presenting a general outline of the principal course in general physiological chemistry, that is, in the elements of normal nutrition, which we have been giving for about six years at the Columbia Medical School, which we have lately

introduced at the N. Y. Teachers' College, and which, I believe, is particularly well adapted for the instruction of all whose interests include an understanding of the principles of general nutrition and dietetics, or any other chemical phase of biology.

In the course to which I allude, we place at the foundation of our experiments and discussions, the chemistry of typical cells as the units of living structure and the essential factors in biological dynamics. Every organism consists primarily of one or more cells. The higher organisms consist not only of cells but contain also much important material that is produced by and in their cells, but which is extracellular in location. Life is so intimately associated with intracellular chemical transformations of energy that it seems to be dependent on, and apparently is an expression of, such intracellular changes. Many of the extracellular changes in organisms are of minor importance in the maintenance of life.

Chemical transformations of energy always involve chemical alterations of substances, *i. e.*, the production of one group of substances from another. Cells consist of mixtures of complex substances, which collectively, during the life of the cells containing them, continuously undergo life-maintaining chemical and physical alterations. Such changes involve the consumption of useful and, to some extent, of necessary intracellular substances, with an attendant production of certain simpler compounds ("waste products") that are of no use to the cells (catabolism). Accumulation of waste produces in a cell interferes there mechanically and chemically with further beneficial transformations. Waste products are promptly ejected from normal cells.

Cells cannot retain their structural integrity, and are also unable to maintain their dynamic capacity, unless the life-giving intracellular processes of consumption and excretion (catabolism) are accompanied or followed by compensatory intracellular processes of repair, or direct replacement, or both (anabolism). This general conception furnishes the key to the whole subject of physiological chemistry. It carries us to the foundation of chemical biology. It emphasizes the cardinal fact that the utilization, removal and replacement of materials by an organism are expressions of its collective cellular requirements and peculiarities.

In this introductory phase of the course to which I am alluding, the composition and constituents of typical cellular masses (protoplasm) are studied; the leading cellular constituents are isolated and their structural as well as their dynamic relationships are indicated, so far as that is possible. This portion of the course is also intended to establish general principles regarding the origin and chemical nature of cellular constituents, the functions of cellular compounds, and the processes that characterize cellular activity and power. Both botanical and zoölogical materials are studied experimentally in these connections.

We then raise the question: How do animal cells obtain the materials required for their "repairs" and for the replacement of the substances consumed and removed in the life-giving transformations, *i. e.*, how are the cells nourished? The answer is found, in part, in an intimate study of the physical and chemical properties of lymph and blood, which are the essential and active intermediaries in the exchange of materials in the animal organism (metabolism). "The circulatory system is the commissariat of the physiological army." The mechanico-chemical processes, by which cells obtain (assimilate) and blood and lymph yield nutrient materials, are considered both from theoretical and practical standpoints. The mechanical and chemical relationships between the cells and the blood, lymph, and "tissue juices" in general, are emphasized. We show that in the exercise of their local absorptive, digestive, transformative and constructive processes, the tissue cells obtain from blood and lymph the materials that are utilized directly or indirectly for the maintenance of cellular structure and the continuance of cellular activities.

Having established the direct nutritional dependence of animal cells upon constituents of the blood and lymph, we lead the student into a study of the substances and processes involved in the renewal of such constituents of blood and lymph as are utilized (assimilated) by the cells. This inquiry is, in effect: How is the supply of nutrients for the cells maintained in the blood and lymph? The chief subjects for consideration in this connection are internal and external respiration, and alimentation and absorption. The oxidative processes and gaseous exchanges involved in respiration are

followed in detail. Under the general head of alimentation we study the composition, digestibility and general nutritive values of typical foods. The various digestive processes are fully treated, the influences of bacteria in the alimentary tract are considered, and the composition of feces, as well as the significance of its chief constituents, is noted. The channels of absorption are followed, and the qualities of the absorbable digestive products, as well as the transformations such products undergo, prior to and after their incorporation into the blood, are given due emphasis.

In the study of alimentation and absorption it is shown, for example, that food, which ordinarily consists for the most part of miscellaneous masses containing many complex substances, is chemically converted into fairly uniform liquid mixtures containing comparatively few, and relatively simple, nutrient products. The student learns that chief among the digestive products are monosaccharids, mainly glucose (representing primarily the food starches), glycerol and fatty acids or corresponding soaps (representing chiefly the food fats), and amino-acids (representing the food proteins). The student is also taught, as clearly as possible in this connection, that the digestive and absorptive processes are analogous, in their results, to the achievements of stone cutters and masons. Stone cutters convert rock masses of various shapes and dimensions into massive construction units, of a few types and sizes, which masons put into harmonious relationships in the erection, repair, or extension of buildings on definite plans. The digestive processes convert organic food substances, of many different types and molecular configurations, into molecular construction units of a few kinds and sizes, which, in most cases, are specially adapted for immediate conversion into compounds characteristic of normal blood and lymph. Various groups of cells along the absorptive channels actively rearrange many of these organic molecular construction units into normal blood and lymph constituents of the more complex types.

The opposite phase of cellular nutrition is next brought forward. How are waste products eliminated from the cells? What are the local and systemic influences and general fate of the various kinds of waste products? The gaseous eliminations having

been considered in the prior study of respiration, and the gastrointestinal excretions having been discussed in connection with the previous observations on feces, further attention (though for the time being only in a general way), is now given to local and general excretion, especially from the skin and kidneys.

This sequence in the consideration of subjects enables us to proceed easily and logically from a study of the typical individual cell, of cells in general, and of the body as a whole, to examinations of specific parts of the organism, *i. e.*, of the groups of specialized cells, the respective tissues and organs. Such examinations develop the essential facts regarding tissue structure and composition, as well as the local and general functional relationships of the peculiar tissue constituents. The relative chemical activities and the corresponding nutritive demands of the tissues as specialized parts are duly emphasized, and the way is prepared for final and more detailed considerations of general metabolism, the nature and metabolic significance of the various urinary constituents, and food requirements and selection.

The laboratory work of this course (5 hours per week for a half year) is extended into every important phase of the general subject. The formal lectures (two weekly), besides correlating the results of the experiments and demonstrations with the essential principles, are devoted to various general themes that cannot be given objective treatment in the time allotted for the course, such as chemical coördinations in the body, the chemical processes in embryonic development and lactation, chemical defenses of the organism against disease, biochemical effects of medicines and other foreign agents, typical biochemical perversions associated with disease, etc. The course is designed to establish fundamental biochemical principles, and to develop capacity and confidence in the interpretation of biochemical phenomena.

The prerequisites of such a course as I have just outlined are physics, general chemistry, organic chemistry, and physiology or general biology. It is impossible to understand the scientific and practical aspects of general nutrition and dietetics without the knowledge afforded by such a course in physiological chemistry.

If my remarks have led you to the conclusion that, in the course

I have outlined, every problem is viewed from its relation to the central fact that the cells are the units of structure and the centers or agents of biological power and activity, one of my main expectations has been realized. It is customary in many quarters, in the study and exposition of the data of normal metabolism, to deal largely in nutritional and dietetic generalities—to consider the body as a whole, but to disregard the chemical peculiarities of its parts and to ignore the eccentricities of local (tissue) chemical behavior. Nutrition and dietetics should be studied and taught, in connection with related subjects pertaining to Home Economics, for the purpose of establishing healthy dietary customs, and thereby preventing malnutrition and consequent disease; and also in order to provide information leading to the relief, and if possible to the cure, of metabolic disorders by adequate nutritional readjustments. Such study and instruction should be based on the fullest knowledge of the peculiarities of cell chemistry in general and of tissue chemistry in particular, both in health and disease.

I am sure you will agree with me when I say that investigations of the kinds and quantities of food supplies shipped into Boston, and inquiries regarding the general selection and preparation of such materials, the main channels of their distribution and the methods of their transportation to the various parts of the city, together with determinations of the nature and peculiarities of Boston's sewage and sewage system, would fail to afford correct or adequate conceptions of Boston's dietary characteristics or requirements. We should have to learn much, besides, about the people of Boston in order to understand and appreciate their collective dietary needs. We might ascertain many general facts pertaining to the city and its inhabitants, in times of peace as well as in periods of disorder, and yet, if we learned little or nothing about the people of Boston as individuals, if we ignored their individual characteristics and activities and needs, as well as the occupations, customs and other peculiarities of the various groups of citizens, our deductions regarding the kinds and amounts of food needed daily in Boston would be decidedly imperfect, and our conclusions on ways and means for maintaining the nutritional welfare of all the people in Boston would be largely guesswork.

So it may be with certain courses in nutrition and dietetics. Our study and teaching of these important matters should not be confined to the accumulation and interpretation of general statistics, but should include due attention to the dynamic factors in metabolism—the individual cells and the tissues, under both normal and pathological conditions.

Such an elementary course as the one I have outlined, if based on adequate preliminary training in physics, chemistry and biology, offers much to be desired in this connection. I am glad to add, in conclusion, that my experience at Columbia with courses of this kind has given cumulative emphasis to these opinions and convictions.

DISCUSSION

Miss Kinne: I should like to ask Dr. Gies where he would place his course in such a scheme as Dr. Stiles has presented.²

Dr. Gies: It seems to me that, in Dr. Stiles' arrangement of courses, which is a very good one, physiological chemistry should occupy a place beside anatomy and mechanical physiology, or should follow anatomy and mechanical physiology. Naturally all arrangements of courses are matters of practical adjustment, and it is a rare thing that any one course can be fitted ideally into a system of courses. I feel that the leading chemical applications to physiology should be emphasized in connection with studies of anatomy and mechanical physiology. My statement that physiological chemistry is chemistry applied to physiology might be put in another way—that physiological chemistry is chiefly *chemical physiology*. I think that, along with anatomy and physiology, chemico-physiological facts and principles should be given due consideration—and in a separate laboratory course whenever that can be done.

Miss Marlatt: I am concerned with the question as to how much of this biological chemistry can be given to students who have not had work in organic chemistry. I should like to ask Dr. Gies' opinion on that matter.

Dr. Gies: Such a condition offers a very serious predicament. It certainly is true that you cannot satisfactorily teach biological

² Stiles: Journal of Home Economics, 1910, ii, 393.

chemistry to students who do not have adequate knowledge of elementary organic chemistry to begin with. If you endeavor to teach the elements of organic chemistry and the applications of chemistry to biology in the same course, you find it impossible to do justice to the latter subject, and you confuse and disappoint the average student besides. I hope the deliberations of this association will bring about increased requirements and greater opportunities in physics, chemistry and biology at the beginning of the training of women in higher education. You need these three tools in your biological work in household arts and sciences; and they should be as effective in your hands as we try to make them for the students and practitioners of medicine.

SUGGESTIONS TO TEACHERS OF BIOCHEMISTRY

1. A proposed chemical classification of lipins, with a note on the intimate relation between cholesterol and bile salts

JACOB ROSENBLOOM AND WILLIAM J. GIES

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.)

I. A GENERAL CHEMICAL CLASSIFICATION OF LIPINS

The teacher of biological chemistry who aims to speak definitely and with precision regarding the fats, and the substances resembling them ("lipoids"), finds himself in a quandry. He may adopt one of the several current classifications. If he does, he will use terms which, in many cases, convey very little chemical meaning to his pupils at the outset of their work. He may modify an established classification to render it more appropriate to his immediate needs. Such a concession to pedagogic expediency is apt to cause confusion when the classification is found to differ from a conventional grouping in the text books. He may propose and teach a classification of his own. The new classification may be less useful than its predecessors, especially in the estimation of his colleagues.

With possibly such a fate as this awaiting it, we venture to propose the accompanying chemical classification of fats and the many substances related to or resembling them, which collectively can be very appropriately and conveniently called *lipins* (pp. 52-53).

Our classification of the lipins has been in the process of gradual development in our teaching and research here for several years, and has been serving a useful purpose, meanwhile, in both instruction and investigation. It helps investigators, teachers, and pupils to get and to keep their bearings in chemical studies of this great group of substances. Further additions to our knowledge of the lipins will naturally extend the range and accuracy of this classification, an outcome to which we are giving continuous attention.

A PROPOSED CLASSIFICATION OF LIPINS

LIPINS ARE ORGANIC SUBSTANCES OF WIDE BIOLOGICAL DISTRIBUTION. IN PRACTICALLY ALL CASES THEY ARE INSOLUBLE IN CONCENTRATED NEUTRAL SALINE SOLUTIONS. THEY ARE SOLUBLE IN HOT ALCOHOL OR IN WARM ETHER, OR, AS IS USUALLY THE CASE, IN BOTH SOLVENTS.

I. Natural aliphatic lipins

- A. SIMPLE LIPINS. Natural aliphatic fats, waxes, soaps, and also the acids and alcohols (except glycerol) represented in them. Excepting the alcohols, these compounds yield soaps with hot concentrated solutions of caustic alkali, or alcoholate, or both. All of these substances contain carbon, hydrogen and oxygen, but are free from phosphorus, sulfur and nitrogen. (Ammonium soaps are exceptions, of course, in the matter of nitrogen content.)
- a. FATTY ACIDS, such as butyric acid and palmitic acid (formic and acetic acids excepted); various *unsaturated* acids, such as oleic acid and linoleic acid; and hydroxy acids of all these types.
 - b. SALTS AND ESTERS of the aliphatic acids comprising Group I, A, a.
 1. *Soaps*. Inorganic salts, such as potassium caproate and sodium stearate.
 2. *Waxes*. Esters of *aliphatic* mono-hydroxy and di-hydroxy alcohols, such as cetyl palmitate (in spermaceti wax) and myricyl palmitate (in beeswax). (See Group II, B—*carbocyclic* esters.)
 3. *Fats* and fatty ("fixed") *oils*—"Glycerides": Esters of the tri-hydroxy alcohol, glycerol, such as tri-butylin, tri-palmitin, and tri-olein.
 - c. ALCOHOLS (mono-hydroxy and di-hydroxy) of the kinds obtainable from waxes, such as cetyl alcohol, carnaubyl alcohol, and myricyl alcohol.
- B. CONJUGATE LIPINS. Natural compounds of simple lipins with non-lipin substances or radicals. All of these compounds contain carbon, hydrogen and oxygen; and practically all of them contain *nitrogen*. These substances, with those in Group II, A, constitute the group of so-called "lipoids"—a term without a definite chemical basis. (*Protagon* is a mechanical mixture containing several conjugate lipins.)
- a. PROTEOLIPINS. Compound lipins *containing protein radicals* ("lecithoproteins"), such as lecithalbumins and ovovitellins. (These products may be mechanical mixtures of proteins and lipins.)
 - b. GLYCOLIPINS. Compound lipins that are free from protein radicals, but which *contain carbohydrate radicals*, such as the "cerebro-galactosids" represented by phrenosin ("cerebron"). The substances in this group are *free from phosphorus*. (Thudichum's "cerebrosids.") (See Group I, B, d.)
 - c. PHOSPHOLIPINS. Compound lipins that are free from protein and carbohydrate radicals, but which *contain phosphoric acid radicals*, such as lecithin, cuorin and sphingomyelin. (Thudichum's "phosphatids.")
 - d. GLYCO-PHOSPHOLIPINS. Phospholipins which contain carbohydrate radicals, such as carnaubon and various phytolcithins. (Jecorin appears to be a phospholipin-carbohydrate *mixture*.)

II. Natural carbocyclic lipins

- A. STEROLS. Natural terpeno-alcoholic derivatives. All of these substances contain carbon, hydrogen and oxygen, but they are free from phosphorus, sulfur and nitrogen. They resist saponification ("non-saponifiable fat"), but form esters (Group II, B). Leading members of the group are cholesterol, iso-cholesterol, koprosterol and sitosterol. These substances are Abderhalden's "sterins" and, with the conjugate lipins (Group I, B), constitute the group of so-called "lipoids."
- B. ESTEROLS. Natural terpeno-aliphatic waxes; soap-yielding sterol esters (Group II, A), such as cholesteryl palmitate in lanolin and cholesteryl oleate in blood.
- C. CHOLATES. Terpeno-acid derivatives of *hepatic* origin, probably from sterols or sterol radicals (page 56).
- a. CHOLIC ACIDS (and their simple biological salts), such as cholic acid and choleic acid.
- b. BILE ACIDS (and their biological salts), such as glycocholic acid and taurocholic acid.

III. Natural lipins of undetermined constitution

- A. CHROMOLIPINS. Pigments which cannot be saponified and which are comparatively unaffected tinctorially by caustic alkali, such as lipochromes.
- B. MISCELLANEOUS LIPINS. Substances of uncertain qualities or doubtful existence, such as krinosin and bregenin.

IV. Artificial lipins

Many laboratory products such as tri-acetin, lead oleate, cholesterol benzoate, sodium cholesterylolate, strychnin-lecithate, stearyl alanate, creatin-lecithate, and potassium-kephalate.

The foregoing general classification is wide open for improvement. Its adaptability to change in harmony with new chemical discoveries is apparent. We expect to present a *detailed* extension of it in the near future.

II. IS CHOLESTEROL THE MOTHER SUBSTANCE OF CHOLIC ACID AND THE BILE SALTS?

During the development of the accompanying classification of the lipins, the senior author issued the following comment in his mimeographed directions for the laboratory work of the course in physiological chemistry required of first year students of medicine at this University (1909-1910):

"470. **Physical and chemical properties of cholesterol.**—The methods employed in these experiments for the isolation of cholest-

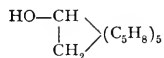
terol have shown its resemblance to fats and lecithins in the matter of solubilities, but have indicated its difference from both fats and lecithins in the matter of saponification. Like fats, but unlike lecithins, cholesterol consists solely of carbon, hydrogen and oxygen. The available data for the percentage elementary composition of most cholesterol indicate that the general molecular nature of the *crystalline* form of the most common member of the cholesterol group is correctly represented by the following empirical formula for that substance (with water of crystallization): $C_{27}H_{44}O, H_2O$. The essential facts in our knowledge of the chemical *constitution* of cholesterol are that these substances (a) are *mono-hydroxy alcohols*, that (b) each of their molecules contains at least one *double bond* (unsaturated), and that (c) the molecules are largely *terpene-like* in structure. The appended *constitutional* formulas have been proposed:



Proposed constitutional formulas for typical cholesterol, $C_{27}H_{44}O$.

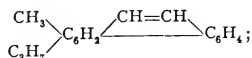
"471. Terpenes occur in many plants, where they appear to be waste products. Terpenes are *hydrocarbons* with compositions indicated by the following general formula: $(C_5H_8)_n$. Each of the molecules of nearly all the terpenes contains at least one 'carbon ring.' One of the familiar oxy-derivatives of terpenes is camphor, $C_{10}H_{16}O$. Terpenes may be regarded as complex benzene derivatives, since camphors and also various terpenes of the type represented by the formula $(C_5H_8)_2$ can readily be converted into cymene, $(C_5H_7)_2$, which is *p*-isopropyl-methyl benzene, $CH_3-C_6H_4-C_3H_7$.

"Each of the above constitutional formulas may be written in more condensed form, to show strikingly the terpene relationships of cholesterol, as follows:



"472. When an alcoholic solution of cholesterol is treated with *d*-methyl furol and concentrated sulfuric acid solution, a pink color is produced and the resultant liquid exhibits characteristic spectral absorption bands. This reaction is very significant in its bearing on the chemical relationships of cholesterol, for it is given also (a) by various derivatives of *camphor*; (b) by *abietic acid*, $C_{18}H_{27}COOH$, one

of the resins which are closely related to the terpenes and may be produced from terpenes by oxidation; (c) by a hydrid of retene,



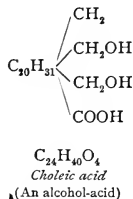
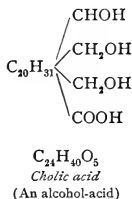
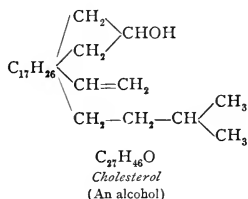
and (d) by *cholic acid*, whose main radical is combined with radicals of glyocol (amino-acetic acid) or other substances in the so-called 'bile acids,' among them *glycocholic acid*,



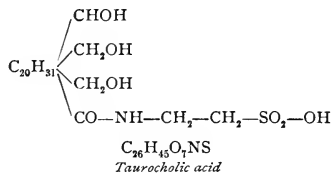
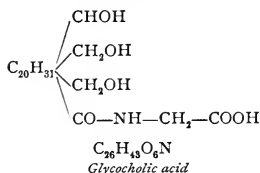
The empirical formula for cholic acid is $\text{C}_{24}\text{H}_{40}\text{O}_5$; that for cholesterol is $\text{C}_{27}\text{H}_{44}\text{O}$.

"The conspicuous presence of both cholesterol and cholic acid compounds in bile, as well as the close resemblance between their empirical formulas and their behavior in the reaction mentioned above, suggests, though by no means proves, that cholesterol is the 'mother substance' of cholic acid and an important factor in 'bile production.'"

There is still considerable uncertainty regarding the empirical formula for cholesterol. That the formula $\text{C}_{27}\text{H}_{46}\text{O}$ is more nearly correct than $\text{C}_{27}\text{H}_{44}\text{O}$, as given above, seems probable from the results of recent researches. The following formulas are now the preferred ones for cholesterol, cholic acid, and choleic acid:



Constitutional formulas for glycocholic acid and taurocholic acid may be written, on the basis of the above graphic formula for cholic acid, as follows:



Cholesterol is a conspicuous constituent of blood corpuscles. Cholesterol esters ("esterols") occur in blood plasma. Bile pigments result from the hepatic decomposition of hemoglobin, and cholesterol is doubtless removed simultaneously from the disorganized erythrocytes. Bile contains soaps, which may arise from the cholesterol esters by hepatic saponification.

Some of the available cholesterol appears to be transformed in the liver, by oxidation in part, into cholic acids. Cholic acids, in turn, are combined in the liver with amino-acids, such as glycocol and taurin, which are present there in abundance. These conjugate acids are further united in the liver with basic elements such as sodium. The "bile salts" that result from these unions dissolve readily in water, physiological salt solution, cell plasm, lymph, blood, and bile. The soaps and bile salts in the hepatic cells favor the solution of associated *unchanged* cholesterol, which accompanies these substances in the bile to the intestine.

Cholic acids and bile salts originate in the liver. Neither cholic acid nor a simple salt of it occurs normally in the blood. The small proportions of bile salts which appear in the circulation are directly or indirectly hepatic in origin. In the light of these additional facts we believe that the above suggestions point the way to an understanding of the biochemical derivation of bile salts.

It is possible, in this view of the probable relationship between cholesterol and bile salts, that cholesterol gall stones arise when, among other causes, the *normal transformation* of cholesterol into bile salts is materially diminished in degree, with a consequent marked increase in the concentration of cholesterol in the bile.

Research along the lines of these suggestions promises interesting and important results.

ORGANIZATION AND EARLY MEETINGS OF THE COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

PROCEEDINGS REPORTED BY THE SECRETARY,

WALTER H. EDDY

I. PRELIMINARY ORGANIZATION, FEBRUARY 1, 1910

At a special meeting of the officers of the Biochemical Department of Columbia University, in the laboratory at the College of Physicians and Surgeons, on Feb. 1, 1910, Dr. Gies suggested and outlined a general plan for the organization of an association which, through local activity and by the publication of a journal, would unite the interest of all past and present, as well as future, workers in the Department of Biological Chemistry of Columbia University and in the laboratories officially affiliated with the Department.¹ Such an association, Dr. Gies suggested, could become an important factor in the increase and extension of biochemical knowledge. In order to formulate these plans into a working agreement, a committee, consisting of Drs. Walter H. Eddy, Matthew Steel and Herman O. Mosenthal, was appointed by Dr. Gies to draft a constitution and instructed to report this to the biochemical staff at a future meeting. On March 1, 1910, at a meeting over which Dr. Gies presided and at which the writer served as secretary, the committee formally presented the appended report.

II. PERMANENT ORGANIZATION, MARCH 1, 1910

Official report of the committee on permanent organization of the Columbia University Biochemical Association.

Mr. Chairman, and fellow workers in the laboratories of the Columbia University Department of Biological Chemistry:

Your committee, which was appointed on February 1, 1910, at the

¹The officially affiliated laboratories are located at the New York Botanical Garden (since 1902) and in the Household Arts Building at the New York Teachers' College (since 1909). The Department was established in 1898.

meeting for the preliminary organization of the Columbia University Biochemical Association, hereby proposes the adoption of the following statement and the appended constitution for the permanent organization and government of our Association:

We, the undersigned workers in the laboratories of the Columbia University Department of Biological Chemistry, in session this first day of March, 1910, hereby adopt the following Constitution for the government of the Columbia University Biochemical Association, which we herewith establish.

Constitution of the Columbia University Biochemical Association

ARTICLE I. *Object*

The Columbia University Biochemical Association has been organized for the purpose of furthering the advancement of the science of biological chemistry through the immediate agency of the Columbia University Department of Biological Chemistry. The association shall aim to interest all past, present, and future workers in the laboratories of the Columbia University Department of Biological Chemistry, and its officially affiliated laboratories, in the attainment of this object.

ARTICLE II. *Membership*

Section 1. Eligibility. A. Any person who has been an *officer* in the Columbia University Department of Biological ("Physiological") Chemistry, or who has been an *investigator*, or has taken a *graduate course*, in any of the laboratories of the said department, or in an officially affiliated laboratory, shall be eligible to membership.

B. Any *under-graduate student*, past, present, or future, in any of the said laboratories, may be elected a member of the association at an annual meeting by unanimous vote of those in attendance.

Section 2. Classification. A. The term *resident member* shall refer, in this Constitution, to every member *officially* connected with the Columbia University Department of Biological Chemistry, or with any of its affiliated laboratories. The term *non-resident member* shall refer, in this Constitution, to members who are without existing *official* connection with the said department or any of its formally affiliated laboratories.

B. Any member, upon the payment of one hundred dollars (\$100), shall become a life member and shall be so designated on all official lists of membership.

C. Any person may be elected to honorary membership by unanimous vote at any meeting.

Section 3. Nomination and election. Nominations for, and elections to, membership may occur at any meeting. A majority of the votes cast shall elect a nominee to membership, except as provided in Section 1 of this Article.

ARTICLE III. *Journal*

Section 1. Quarterly publication. The Association shall publish a Journal to be issued quarterly, or more frequently at the discretion of the Editorial Committee (Article IV, Section 3).

Section 2. Character of the Journal. The Journal shall contain departmental news, personalia, abstracts of research done by members, and such other matter as the Editorial Committee may select, or the Association may determine.

ARTICLE IV. *Officials*

Section 1. Officers. The officers of the Association shall be an *Honorary* President, five *Honorary* Vice-Presidents, a President, a Vice-President, a Secretary and a Treasurer.

Section 2. Executive Committee. The Executive Committee shall consist of the Head of the Columbia University Department of Biological Chemistry, the President, Vice-President, Secretary and Treasurer of the Association, and enough additional elective members to make a total of seven. The Vice-President shall be the chairman of the Executive Committee.

Section 3. Editorial Committee. The Editorial Committee in charge of the Journal shall consist of the Head of the Columbia University Department of Biological Chemistry, the President, Secretary and Treasurer of the Association, and enough additional members to make a total of thirteen. The Secretary of the Association shall be the chairman of the Editorial Committee and the Editor-in-chief of the Journal.

Section 4. Council. The Council shall consist of the Executive Committee and the Editorial Committee. The Vice-President shall be the chairman of the Council.

Section 5. Nomination and Election: A. Nominations of officials shall be made by the Council at least one month before the annual meeting. Additional nominations may be made by members at the Annual Meeting.

B. Election. Election of officers shall be by ballot at the Annual Meeting. Non-resident members shall be eligible to vote by proxy. A plurality of the votes cast shall elect.

Section 6. Term of Office. The term of office shall be one calendar year.

Section 7. Duties. The duties of the officers shall be such as usually devolve, individually and collectively, upon their respective offices, besides such obligations as the Association may specifically impose.

ARTICLE V. *Meetings*

Section 1. Time and place. The Association shall hold at least four meetings during an academic year, at such times and in such places as shall be designated by the Executive Committee, except as provided in Section 2 of this Article.

Section 2. Annual Meeting. The Annual Meeting shall be held in New York City during Columbia University Commencement Week.

ARTICLE VI. *Dues*

The annual dues for all *resident* members shall be One Dollar (\$1.00), payable on or before May 1.

ARTICLE VII. *Quorum*

Seven members shall constitute a quorum for the transaction of business.

ARTICLE VIII. *Amendments*

This constitution may be amended at any time by a three fourths vote of the *resident* members.

Respectfully submitted,

WALTER H. EDDY,

MATTHEW STEEL,

HERMAN O. MOSENTHAL,

Committee.

Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, February 28, 1910.

The foregoing report was unanimously approved. The Association was thereupon permanently organized, March 1, 1910, with the adoption of the above constitution for its government.

The following members (9) were present at this meeting of permanent organization: Stanley R. Benedict, Walter H. Eddy (Secretary), William J. Gies (Chairman), Isidor Greenwald, Alfred P. Lothrop, Herman O. Mosenthal, Jacob Rosenbloom, Emily C. Seaman, and Matthew Steel.

Officers. At the same session the following officers were elected to serve until the first annual meeting (June, 1910):

Honorary President: Alfred N. Richards, Professor of Pharmacology in the Northwestern University Medical School, Secretary of the American Society of Biological Chemists, and Assistant Editor of the Journal of Biological Chemistry.

Honorary Vice-Presidents:

P. B. Hawk, Professor of Physiological Chemistry at the University of Illinois, and author of "Practical Physiological Chemistry."

C. Stuart Gager, Professor of Botany, University of Missouri.

William Salant, Pharmacologist, U. S. Department of Agriculture.

Wm. H. Welker, Demonstrator of Physiological Chemistry, University of Pennsylvania.

Raymond H. Pond, Plant Pathologist, Texas Agricultural Experiment Station.

President: Nellis B. Foster; *Vice-President:* Herman O. Mosenthal; *Secretary:* Walter H. Eddy; *Treasurer:* William J. Gies.

Additional members of the Executive Committee (resident members): Stanley R. Benedict, Matthew Steel, Alfred P. Lothrop.

Additional members of the Editorial Committee (local, non-resident members): Leo Buerger (Mt. Sinai Hospital), Arthur F. Chace (Post-Graduate Medical School), Louis J. Curtman (New York City College), Louis Hussakoff (American Museum of Natural History), Gustave M. Meyer (Rockefeller Institute), Raymond C. Osburn (Barnard College), Jacob Rosenbloom (German Hospital), Emily C. Seaman (Teachers College), Fred. J. Seaver (New York Botanical Garden), William Weinberger (Lebanon Hospital).

III. FIRST ANNUAL MEETING, JUNE 3, 1910

Unexpected difficulties stood in the way of formal sessions of the association before the inauguration of the series of scientific meetings now in regular progress, but the first annual meeting was

held at the appointed time—on June 3, 1910, at the College of Physicians and Surgeons. In the absence of President Foster, the Vice-President, Dr. Mosenthal, presided.

Officers. The only matter of public interest in the proceedings of this meeting was the election of the following officers (1910-11):

Honorary President: Alfred N. Richards, Professor of Pharmacology at the University of Pennsylvania, Secretary of the American Society of Biological Chemists, and Assistant Editor of the Journal of Biological Chemistry.

Honorary Vice-Presidents:

P. B. Hawk, Professor of Physiological Chemistry at the University of Illinois, and author of "Practical Physiological Chemistry."

William Salant, Pharmacologist, U. S. Department of Agriculture.

*Stanley R. Benedict, Assistant Professor of Chemical Pathology, Cornell University Medical College.

*Matthew Steel, Assistant Professor of Dairy Husbandry, University of Missouri.

Raymond H. Pond, Plant Pathologist, Texas Agricultural Experiment Station.

President: Nellis B. Foster; *Vice-President:* Herman O. Mosenthal; *Secretary:* Walter H. Eddy; *Treasurer:* William J. Gies.

Additional members of the Executive Committee (resident members):

*Wm. H. Welker, *Jacob Rosenbloom, Alfred P. Lothrop.

Additional members of the Editorial Committee (local, non-resident members): Leo Buerger (Mt. Sinai Hospital), Arthur F. Chace (Post-Graduate Medical School), Louis J. Curtman (College of the City of New York), Louis Hussakoff (American Museum of Natural History), Gustave M. Meyer (Rockefeller Institute), Raymond C. Osburn (Barnard College), Emily C. Seaman (Teachers College), Fred. J. Seaver (New York Botanical Garden), Wm. Weinberger (Lebanon Hospital).

IV. FIRST REGULAR SCIENTIFIC MEETING, FEBRUARY 28, 1911

The first regular scientific meeting of the Biochemical Association was held on Feb. 28, 1911, at the College of Physicians and Surgeons. Dr. Gies presided. About thirty members were pres-

* Newly elected officer.

ent. The Association was the guest on this occasion of the Staff of the Columbia University Biochemical Department. The scientific proceedings consisted of a symposium on "The Chemistry of the Cell," which was conducted by members of the Biochemical Staff, with the following program:

- I. Introduction, including remarks on water in cells. . . Wm. J. Gies
- II. Salins William H. Welker
- III. Carbohydrates Ernest D. Clark
- IV. Lipins Jacob Rosenbloom
- V. Proteins Walter H. Eddy
- VI. Extractives Isidor Greenwald
- VII. Enzymes Alfred P. Lothrop
- VIII. Factors in immunity Reuben Ottenberg

In his introductory remarks after taking the chair, and before opening the symposium, Dr. Gies expressed the very great personal pleasure it gave him to preside at a meeting which was so significant, and which was attended by so many of his past and present co-workers. He drew attention to the unusual character of the Biochemical Association and spoke of its opportunities for special service. He welcomed the happy occasions these regular scientific meetings would afford for the frequent reinvigoration of old friendships as well as for the formation of new ones. He was gratified to believe, from the interest in this initial meeting of the contemplated series, that the Association would stimulate further growth of the "family spirit" which has always been happily conspicuous among the workers in biological chemistry whom it has been his good fortune and happiness to guide. Dr. Gies expressed both the hope and the conviction that the Biochemical Association would be a source of great personal and professional profit and pleasure to all its members and that it would have an honorable history and enjoy a useful career. He invited the cooperation and good will of all to these ends.

The hearty approval which was accorded to these sentiments gave strong evidence of the existing earnestness of purpose and harmony of feeling among the members of the association.

General discussion of "The chemistry of the cell," which was

led in very instructive manner by Drs. Foster and Mosenthal, terminated the scientific proceedings. Refreshments were then served and an enjoyable social session was continued until long after midnight.

The abstracts of the papers comprising the symposium are given on pages 65-93.

(The proceedings of the June meeting of the Biochemical Association will be published in the December issue of the BIOCHEMICAL BULLETIN.)

CHEMISTRY OF THE CELL

Abstracts of the communications which comprised the symposium, on the chemistry of the cell, at the first scientific meeting of the Columbia University Biochemical Association, held at the College of Physicians and Surgeons, on Feb. 28, 1911.

I. INTRODUCTION, INCLUDING REMARKS ON INTRACELLULAR WATER¹

William J. Gies

Introduction. Joint histological and chemical study makes it evident that every living organism consists primarily of one or more CELLS containing mixtures of substances undergoing continuous, coordinated chemical and physical changes. Cells are the microscopic units of bioplasmic structure. As the so-called "physiological units," cells are the centers of biochemical activity, the seats of biological power, the locations of vivifying energy transformations, and the dynamic agents in structural enlargement, in repair, and in reproduction. Cells are biophysical factors in consciousness and mental phenomena.

Every organism begins its existence as a single cell. Heredity, reproduction, embryonic development, tissue production, growth, repair, regeneration, maintenance, metabolism, body temperature, motion and locomotion, conduction of stimuli, responsiveness to stimulation, resistance to and recovery from disease, mentality, etc., are expressions of intracellular forces as affected by inherent impulses and by both intracellular and extracellular influences. Cells are specially and variously constructed, as well as definitely associated, *mechanical* agents in life phenomena. Cell constituents are intimately coordinated and functionally adjusted *chemical* factors in all the manifestations of life. The structural

¹Informal introductory remarks are abstracted on page 63. In the preparation of the first two abstracts, free use has been made of the author's comment in the laboratory directions for his courses in physiological chemistry.

as well as the dynamic properties of a cell are dependent upon the qualities, conditions, interrelationships, and proportions of its chemical constituents.

Cell problems are embodied in all other biological questions. What are the constituents of living cells of any particular type? How are these constituents, as we now know them chemically, coordinated with or related to each other? To what degree is any particular vital phenomenon due to the chemical or mechanical heterogeneity of the contents of the controlling cells? To what extent do specific intracellular physical and chemical relationships, individually or collectively, affect the functional powers and peculiarities of a cell? Which cellular phenomena are due to adsorption, which to surface tension? How far may these intracellular chemical situations be disturbed without impairment of a cell's activities? Which cellular functions are influenced by intracellular osmotic forces? Which cellular phenomena are collochemical? What general or local effects accrue from "spontaneous" rearrangements of cell constituents? What new chemical and physical coordinations result within a cell from the influence of unusual conditions without? What are the intracellular foundations for the tropisms? What is the exact nature of a given cell "membrane" and what are the functional powers of such membranes? To what extent is disease an expression of disadvantageous coordinations of cellular constituents in response to unfavorable influences? To what degree is successful resistance to disease the result of a capacity to effect defensive intracellular alinements of chemical constituents? Which symptoms of a given disease arise from the incapacity of a particular group of cells to readjust their internal chemical parts for the maintenance of adequate protection against disturbing external agents? Medicinal as well as toxic effects ensue through influences on cell constituents but how, in a given case, are the effects induced and upon which of the constituents, and why, are they thus primarily registered? How do nutritive excesses or nutritive deficiencies affect intracellular conditions? How do mental and emotional conditions bring about the profound influences so often exhibited through both mechanical and chemical

changes in cells? Why, in turn, do certain discoordinations and new relationships among cell constituents induce the effects which are frequently seen in the many well known nervous disorders and temperamental derangements?

Dozens of questions of similar import come to mind in any thoughtful consideration of the relationships of cell constituents to each other and to cell vitality and functions, yet who is able to answer any one of the foregoing questions in terms of our present meagre knowledge in this field. It was long-standing appreciation of the fundamental biological importance and the practical medical significance of research in this broad and open field, that led me, in the fall of 1909, after a number of studies of protein salts, to propose the series of investigations "*on the composition of protoplasm and the nature of the structural and dynamic relationships of cell constituents and products*," which was then inaugurated and which has since been in progress in our laboratory under the auspices of the George Crocker Special Research Fund. This proposed series of chemical investigations has the great merit of being broadly fundamental in *biological* character, essential in *physiological* nature, significant in *pathological* bearing, and promising in its ultimate crop of facts and principles relating to the *etiology of cancer*.

Ehrlich has shown that the defensive agents "at the seat of war" in cellular pathology are special *substances* and *molecular groups* of intracellular derivation. *Diagrams* illustrative of the details of Ehrlich's great conception, and showing the "toxophore groups," "haptophore groups," "zymophore groups," "cytophile groups," "receptors," "amboceptors," "complement," "toxins," "antitoxins," "agglutinin," etc., etc., appear in every up-to-date book on pathology. The investigator of immunity problems thinks in terms of these diagrams and the student of immunity learns the fundamental views in terms of such figures. Yet all of the Ehrlich "bodies" are figurative and very useful makeshifts for important realities of intracellular constitutions, cellular affinities, extracellular dynamics, and the reactions of cell constituents with extracellular substances. How much more clearly all of it could be put, and how much more effectively the facts could be used, if the Ehrlich theories were based on exact *knowledge* of intracellular chemical

conditions rather than upon *hypotheses* relating to them only in gross.

Intracellular water. When cells are completely desiccated at the temperature of boiling water, they may lose by volatilization more than 50 per cent. of their weight. Some cells lose as much as 98 per cent. of their weight under such conditions. The volatile matter consists almost wholly of water, which is quantitatively the leading constituent of living cells. Most of the water in an organism is, as a rule, intracellular in location.

The temperature of boiling water destroys the life of every known type of cell. This effect is evidently due, in great degree, to influences arising from abnormal vapor tension, excessive hydrolysis, destruction of enzymes, and solidification and consequent discoordination of the coagulable colloids, which, next to water, comprise the largest proportion of intracellular material. A moderate degree of desiccation of most animal cells at their normal temperatures results in modified function, altered structure, impairment of vitality, or death, or all of these, according to the proportion of water remaining in the cells. Water-bears are among the few animals which may be dried and thus rendered apparently lifeless, but whose form and vital activity are recovered with immersion in water. Vegetable cells of many types, including bacteria, may be thoroughly desiccated at biological temperatures, until they seem no longer to possess any of the characteristics of living matter. Yet the life activities of such dry cells may be only temporarily arrested, *i. e.*, the cells may be potentially alive. In such cases the cells, after remaining for months or even years in a state of dormant vitality, may be quickly rendered actively alive, if an adequate amount of water is supplied.

Refrigeration, as in the case of high temperature (and for similar reasons), destroys the life of most types of cells, but cells in many of the simpler forms of organisms withstand the destructive influence of solidification of the water in them. The larger the proportion of intracellular water, however, the greater the deleterious effects of freezing appear to be. Destructive effects from the freezing of intracellular water are doubtless due very largely to the abnormality of the new phase relationships thus created in the heterogeneous mass of which each cell is composed.

The foregoing statements emphasize the further fact that cells differ greatly in their immediate need of water, in their resistance to its intracellular removal, and in their power to withstand the changes in its physical condition which are induced by alterations of temperature. Water is an essential "nutrient" at all periods of a cell's life. The largest proportions of water commonly occur, in mammals, in the tissues containing the most active cells.

Hydrotropism, which is one of the important manifestations of life, is cellular response to the stimulating influences of water—a typical chemotropic reaction. The elastic rigidity of many plants with large proportionate contents of water is due to the tension of their cell walls under the influence of intracellular osmotic pressure, colloidal affinity for water, and other forces.

Water may readily be withdrawn from cells by simple diffusion into such dehydrating agents as alcohol. Water continually evaporates from cells in superficial locations. In plants "wilting" is an indication of such a loss of water and a consequent reduction of turgidity. The presence of water in cells can be immediately demonstrated colorimetrically with such a dehydrating agent as anhydrous copper sulfate.

Our general knowledge of the facts regarding the condition and significance of intracellular water warrants the following additional summary:

Cells contain large proportions of water in the *free* state. Free intracellular water may be readily removed by evaporation, by ordinary diffusion, and by dehydrating agents. Intracellular water is the general "medium of exchange" inside the cell, and also between the cell and its liquid environment. This free intracellular water contains the soluble intracellular compounds. It is therefore the mobile medium through which osmotic forces are manifested, which provides for the ingress and egress of diffusible substances (nutrients and waste products particularly), which cooperates in maintaining the functional integrity of the cell colloids, and which provides the carrier for all the vital reaction-exchanges. Intracellular water is normally in comparatively stable equilibrium with (A) the water among the adjacent tissue cells, (B) the water in the

circulatory system and (C) the water in the secretions and excretions. Any disturbance of this equilibrium, particularly by special loss from the whole organism, is speedily felt in the individual cells.

Much of the intracellular water is consumed to local advantage in the chemical processes characteristic of any given type of cell. Some of the water in a cell appears to unite molecularly, especially with colloids, in forms comparable to water of crystallization (hydration). Water is directly involved in various intracellular processes of hydrolytic dissociation, hydrolysis (hydrolases), and oxidation (oxidases). Free water is also *produced* in cells, especially by intracellular processes of dehydration and oxidation.

Water is certainly an important factor in essential intracellular phenomena of surface tension. It seems to participate in complex intracellular unions of masses and molecules which are peculiar to protoplasm. That there are many gaps in our knowledge of the relations between intracellular water and many other cell conditions and phenomena, is very evident to all who interest themselves in this perplexing subject. What a fruitful field for research!

II. INTRACELLULAR SALINS

William H. Welker

The ash that results from the incineration of cellular matter is neither a true qualitative nor an exact quantitative representation of the inorganic matter occurring in the material. Incineration causes volatile inorganic substances, such as chlorid, to disappear in part. On the other hand, elements such as iron, carbon, and sulfur, in true organic combinations, are oxidized in great degree to non-volatile inorganic products. Then, too, incineration induces decomposition of and reactions among intracellular inorganic constituents. Such changes may cause loss by volatilization and also induce alterations in the nature of some of the residual inorganic radicals.

Aqueous extracts of cellular matter yield less ash than the corresponding amounts of the tissue itself. Dialysis experiments now in progress in this laboratory (by Erpf-Lefkovich under Dr. Gies' direction) indicate that the quantities of inorganic matter which can be removed from cellular materials by long continued diffusion

are less than those obtained from equal amounts of the same material by incineration. It is probable that in this process there is a certain degree of *production* of free inorganic molecules (ions), especially as a result of hydrolytic dissociations of complex intracellular compounds of organic-inorganic types. These dialysis experiments have been undertaken in due appreciation of the present paucity of knowledge regarding the nature of the intracellular inorganic substances and are being continued in the hope of increasing our information along these fundamental lines.

Our general knowledge of the conditions and characters of intracellular inorganic substances is stated briefly in the following summary.

All cells require inorganic salts for their growth and maintenance, and for the exercise of their vital activities. Cells speedily die when all inorganic salts are continuously denied them, however favorable all the other conditions for cellular existence may be. Cellular activities are rendered pathological, when the equilibrium among the intracellular salin substances is materially disturbed by an abnormal increase or decrease in the proportion of any one salin substance or ion.

All cells yield inorganic substances by incineration, by simple extraction and by dialysis. Of the inorganic mass obtained by direct incineration, or by burning extracts or diffusates, much is pyrogenous, as has already been stated, and, therefore, cannot truly represent intracellular inorganic matter. Of the inorganic masses obtained by extraction and dialysis, some is apparently produced from complex intracellular compounds by hydrolytic dissociation.

Some of the intracellular inorganic matter seems to occur in the form of ordinary salts. A small proportion of this simple salin matter, such as calcium phosphate, may be suspended in the intracellular mass, but most of the true salin material appears to be dissolved in the intracellular water, where the salts evidently exist in molecular forms and also in ionized states. This plain inorganic matter is characterized biologically by its mobility, its osmotic power, and its readiness to enter into new chemical reactions of great functional import under the influence of changing extracellular as well as intracellular conditions.

“Organic combinations” of inorganic materials occur in cells in

a number of important forms, of which the following types seem to be the most common:

1. Labile compounds, consisting of *molecular* combinations similar to glucose-sodium chloride, and *atomic* combinations, such as sodium-proteinate. These compounds readily undergo both hydrolytic and electrolytic dissociation.

2. Comparatively stable compounds, consisting of inorganic *radicals* and *atoms* incorporated in organic molecules, such as the radicals of phosphoric acid in lecithins and the atoms of iron in nucleoproteins. These compounds do not appear to undergo hydrolytic dissociation.

The "organic combinations of inorganic matter" are, as a class, when compared with the simple salin constituents, less mobile, less osmotic, and less prone to enter reactions in response to fluctuating extracellular and intracellular conditions.

That there are many types of adsorption products between intracellular inorganic substances and colloids is obvious from the work already done by many investigators.

It is very probable that protoplasm consists of, or at least contains, highly involved combinations of representatives of both the simple and complex types of compounds mentioned above. We have very little definite chemical knowledge on this point, however. The story of the functions of the intracellular inorganic compounds, radicals and atoms, must be a long and instructive one, yet even the introductory chapter of it cannot as yet be written with definiteness or satisfaction.

III. INTRACELLULAR CARBOHYDRATES

Ernest D. Clark

The leaves of the higher plants contain the most efficient means of changing the radiant energy of sun-light into the potential energy of carbohydrate. The chloroplast of the plant cell is the primary agent in this important process. The animal cell is essentially a transformer of stored energy into work and heat. On that account we should expect that the carbohydrates would not be plentiful at any time in animal cells and that carbohydrates would not long exist there in their original forms, although much carbohydrate might be consumed in them. Such is the case, for in animals most

of the carbohydrate is in the process of downward transformation, whereas in the plant carbohydrate is synthesized in large quantity and much of it is stored in complex forms which may readily be identified. Besides acting as energy stores, some carbohydrates probably supply radicals and atomic groups for the intracellular synthesis of proteins and fats. The carbohydrates seldom figure as conspicuous parts of animal cells but in plants the contrary is true; in fact, the walls of most plant cells are composed of cellulose or related carbohydrate materials.

Free carbohydrates. Of the carbohydrates which exist *uncombined* in the PLANT cell, the most abundant and best known are the starches. *Starch* exists in the storage organs in the form of characteristic grains which often fill the cells of such tissue with granules of various sizes. In the cells of the leaf where the starch is being formed, the grains are attached to the chloroplasts, which are the active agents in photosynthesis. *Inulin* is another storage carbohydrate somewhat like starch but differing in the fact that inulin remains dissolved in the cell-sap of the storage organs of the Compositae and is precipitated in spherocrystals in the cell upon the addition of alcohol. *Glycogen* which is so important as a carbohydrate store in the animal body is not often found in the higher plants but it occurs in bacteria, forms granules in yeast cells, and also appears among the fungi in the form of highly refractive microscopic masses. In certain oral bacteria, grains have been noted which stained blue with iodine, but it has never been proven that these grains were really composed of ordinary starch. Among the carbohydrate reserves in other plants are *sucrose*, especially conspicuous in the sugar-cane and sugar-beet; also rarer sugars, like *raffinose*, *rhamnose*, and *gentianose*, all of which are found free in the cell-sap of the plants after which many of them are named. *Maltose* is present most noticeably in the cells of germinating seeds where the starch has been partially digested by the diastase. *Glucose*, *fructose*, and other hexoses occur in the cells of most plants, functioning either in the destructive metabolism of the plant or as construction units for the other substances synthesized by the plant. It is an interesting fact that the *pentoses* rarely occur free in cells but only in the form of nucleoproteins, cell-wall constituents and the like.

Starch is the most important storage carbohydrate in plants. Glycogen plays a similar part in ANIMALS. Glycogen occurs most conspicuously in the liver, in smaller proportions in muscles, but to some extent in all animal cells and organs.

The blood receives a steady supply of glucose from the alimentary tract yet the glucose content of the blood is practically constant. The glucose in the blood readily enters the body cells and supplies them with calorific material and substance for advantageous coordinations and constructions. Maltose may also be mentioned as another free sugar in animal cells, but it exists only in traces, probably arising from glycogen. *Lactose* is conspicuous in mammary cells during lactation.

Combined carbohydrates. Besides the carbohydrates which are free in the PLANT cell there are other important substances which contain the sugar groups in *combined* condition. The *glucosides* are noteworthy examples of this type. Glucosides consist, in most cases, of sugar (hexose) united with another substance, usually of the carbocyclic series. Glucosides are often held in their own special cells in storage until a rupture of the cells occurs, then the hydrolytic enzymes of neighboring cells attack and rapidly resolve the glucosides into their sugars and cyclic constituents. The sugars in glucosidal substances act as a reserve supply of carbohydrate and the associated part is often an easily oxidizable material, or one having a striking odor, peculiar color, antiseptic properties and so on. The role of the glucosides in the plant is not known, but the widely diverse nature of their component parts makes it likely that the latter are important factors in the plant's life processes. The *nucleoproteins* comprise another large class of combined carbohydrates, and contain hexose or pentose radicals. The origin of the nucleoproteins in the cell nucleus indicates that they are intimately involved in the phenomena of heredity. Practically all plant *lecithans* already prepared have contained considerable amounts of reducing sugars, mostly hexoses. Whether such sugars are constituent parts of lecithan molecules, or merely accidental impurities in the products, cannot be said with certainty at present.

Among the combined carbohydrates of ANIMAL cells nucleoproteins may be mentioned as being important in many ways, as has

already been indicated. Aside from the nucleoprotein combination, carbohydrate also exists in cells combined with protein in the form of glycoproteins. These substances are usually produced by special cells and are formed along with other secretions, as in the case of saliva. The brain and nervous system of the higher animals contain complex compounds called cerebro-galactosides. In these lipins we often find galactose-yielding radicals are united to fatty acids groups and also to nitrogen and phosphorous derivatives. Finally, glucose appears in a somewhat similar combination called jecorin, which occurs in the liver, heart-muscle, and other organs. The nature of jecorin is unknown and further work upon it is very desirable.

Intracellular carbohydrates are freely soluble in the associated water; some are insoluble in it. The soluble intracellular carbohydrates are diffusible, and easily pass back and forth in the cells and through the cell "membranes." Although they are comparatively stable substances, the intracellular carbohydrates combine with oxygen, with water, with salins, with carbohydrates, with lipins, and with proteins. That intracellular carbohydrates are involved more actively in intracellular dynamics and reconstructions than I have here indicated is a belief which all of us share but on which none of us is able as yet to say very much that is definite.

IV. INTRACELLULAR LIPINS

Jacob Rosenbloom

In this discussion I shall refer to lipins in terms of the classification given on page 52.

It is well known that *normal* organs like the heart and kidneys will show no evidence of fat, on section, whereas chemical analysis may indicate that 15 to 20 per cent. of the dry weight of the organ is "fatty" in nature. This "masked" fat is combined with other constituents of protoplasm. In *pathological* organs which show microscopically the presence of an abundance of fat, the total amount of fat may be raised above the normal. As a result of certain postmortem or acute changes in such cases, the fat which normally is "masked" is liberated from its combinations and

thus becomes evident. This circumstance shows one of the many important reasons for understanding the way in which "lipins" are held and coordinated in cellular material.

In this discussion of the lipins that occur in cellular material, mention will also be made of certain products of a synthetic nature that bear on intracellular problems.

Compounds of lipins with inorganic substances and tissue metabolites. It has long been known that the complex lipins contain inorganic elements, but at present it is not always possible to say whether such elements are held in mechanical mixtures, or by true chemical unions, or as adsorption associates. The importance of such relationships in intracellular phenomena may be very great, whether they are adsorption unions or true chemical combinations. Koch has lately determined the characters and proportions of the inorganic elements in various preparations of lecithin and kephalin. He found considerable potassium in kephalin and thinks that the abundance of potassium in cells is due to the affinity of potassium for kephalin. Koch has also shown that hypoxanthin, creatin, creatinin, adrenalin, and ammonium salts combine with lecithin. Winternitz has found calcium to the amount of 6.7 per cent. in a vegetable phospholipin. Lecithans unite chemically with both acids and bases.

Compounds of lipins with amino-acids. Abderhalden and his co-workers have prepared compounds of glycin, alanin, and tyrosin with stearin and with palmitin; also compounds of glycerol with tyrosin, and glycerol with palmitin and tyrosin. They have also described compounds of cholesterol with amino-acids and with fatty acids. That similar compounds occur in protoplasm is very probable.

Compounds of lipins with proteins. The substances of this nature which have received most study are the "lecithalbumin" of Liebermann and the "ovovitellin" of Hoppe-Seyler. Liebermann obtained protein products containing lecithin as an insoluble residue after peptic digestion of the mucous membrane of the stomach, liver, kidneys, lungs, and spleen. He considered these residues to be true *compounds* of protein and lecithin. It is impossible to remove the lecithin from this combination with ether or cold alco-

hol, but this is also true of artificial mixtures of protein with lecithin. Hoppe-Seyler supposed lecithin to be combined with protein in the vitellin of egg yolk. Beyond the fact, however, that the lecithin in the yolk could not be extracted from ovovitellin by ether, but was extracted by hot alcohol there is no evidence to show that lecithin and protein form definite chemical combinations in egg yolk. Hoppe-Seyler also regarded "ichthulin" as a lecithalbumin.

Osborne and Campbell found in egg yolk a substance that was soluble in salt solution and which consisted of a mixture of compounds of protein matter with lecithin. These compounds contained from 15 to 30 per cent. of lecithin and they thought these substances might be called lecithin nucleovitellin. It is also interesting to note that Hoppe-Seyler believed that chlorophyll is a lecithin derivative in which the fatty acid radicals are replaced by chromophoric groups of acid character. These groups he called chlorophyllanic acid. However, Schunck and Marchlewski, and Stoklasa, have thrown doubt on this assumption.

Some of the phospholipins in which the relation of nitrogen to phosphorus is 2: 1, *i. e.*, the so-called diamino-monophosphatids, are soluble in ether, but cannot be directly extracted from cells by ether. They can be withdrawn by ether after preliminary treatment of the tissue with alcohol. It is therefore possible that these substances exist in the tissues in combination with protein. This view is strengthened by the fact that tissues contain a certain amount of fat, which can only be extracted after peptic digestion.

Joachim has described a compound of lecithin with pseudoglobulin from a chylous fluid. Brown and Morris, in a study of anemia produced in dogs poisoned with monoacetyl-phenylhydrazine, observed the presence of a substance in the serum which gave the serum a milky appearance, but on addition of various fat solvents, no fatty matter could be dissolved out. However, after the addition of a small amount of ammonium oxalate to precipitate the calcium, a large amount of fatty matter could then be extracted from the serum by means of ether. Brown and Morris have therefore concluded that the fat was combined in the nature of a calcium protein-compound.

Compounds of lipins with carbohydrates. Drechsel de-

scribed a substance containing nitrogen, sulfur, and phosphorus, which he prepared from liver and called jecorin. It has since been studied by Baldi, Baskoff, Manasse, and others. Baskoff obtained very constant figures for its sugar content in a number of different preparations and also found that its nitrogen-to-phosphorus ratio was constant. Bing, Mayer, and others, contend that jecorin is a mixture of lecithin and glucose. Mayer and Terroine think it is formed by the simultaneous precipitation of glucose and lecithalbumin.

The true glycolipins, such as cerebro-galactosides, are definite chemical substances of a fatty nature containing galactose-yielding radicals. Certain "complex" lipins from vegetable material have been shown to yield as much as 16 per cent. of galactose.

Berthelot has described the synthesis of carbohydrate esters of fatty acids and Bloor prepared a compound of mannite with fatty acids.

Compounds of lipins with alkaloids. Bing has described the preparation of compounds of various alkaloids and glycosides with lecithin. Koch has lately shown that various alkaloids unite with lecithin. He has drawn special attention to the affinity of strychnin for lecithin. There is also reason to believe that the constituents of digitalis form chemical products with complex lipins of heart muscle.

Compounds of lipins with toxins. It is well known that the glycolipin known as phrenosin ("cerebron"), which is obtainable from brain, has the power of neutralizing a large amount of tetanus toxin, but whether this is a chemical or physical effect of the phrenosin is still debated. Cobra venom has active hemolytic powers; but corpuscles of certain animals washed free from serum are not hemolyzed by it unless something that can be obtained from the serum which is soluble in ether or in alcohol, be added. Since the addition of lecithin enables the venom to act on washed corpuscles, Kyes regards the hemolytic agent as a combination of the toxin with the lecithin present in the serum. He thinks a definite chemical compound, which he calls cobralecithide, is formed—a compound in which a fatty acid radical of lecithin is replaced by the venom hemolysin. Bang, however, thinks this view

is incorrect, and that the venom merely absorbs the hemolysin.

General conclusion. This brief review emphasizes the meagre nature of our knowledge concerning the way in which lipins are held and coordinated in cellular material, and emphasizes the necessity for further work along these lines—especially the need for new methods of attacking the problem.

V. INTRACELLULAR PROTEINS

Walter H. Eddy

To biologists in general the cell is the essential unit of living matter. The biochemist is apt to think of it in terms of the materials he can obtain from it. He classifies them into primary and secondary constituents, and investigates each as a unit of greater or less interest. The morphologist thinks of the cell in terms of its structures and is interested in tracing the development of these. The physiologists are concerned primarily in the activity of the cells. All, however, unite in general interest in the proteins and their significance in the cell.

The following tabulation shows how morphology and biochemistry meet in considering the cell proteins:

Morphological cell units	Proteins composing same.
Cytoplasm	Albumins, globulins, etc.
Nucleoplasm	Nucleoproteins.
Cell membranes	Keratin, elastin, chitin, gelatin.
Chromatin	Nucleoproteins ranging through nuclein to pure nucleic acids.
True nucleoli	Phosphoproteins.
False nucleoli ("net-knots") ..	Nuclein.
Linin	Phosphoprotein (synonyms: nuclealbumin, plastin, parachromatin, etc.).
Centrosomes	Phosphoproteins.
Nuclear membrane	Sometimes nuclein, sometimes phosphoproteins.

Kossel, Heidenhain, Ehrlich, and others, have spent much time in elucidating the relation of staining reactions to these protein con-

stituents and chemistry has made clear many points in the physiology of these units. It is true that in general the chemist has studied these compounds by extracting cells in mass, whereas the histologist is more concerned with their identification in the cell itself; but the work of one supplements that of the other.

Another purely biological problem which enlists the efforts of the biochemist is the problem of heredity. We know that the chromosomes are the carriers of the hereditary characters and that they are rich in nuclein or nucleic acid, but the nature of the particular particles of these chromosomes which convey the characters is evidently a problem in their chemical arrangement. Nageli's classification of idioplasm as "heredity-conveying" protoplasm and his idants or chromosomes, ids or visible chromatin granules, idioplasts or pangens, determinants or invisible molecular aggregates, is an example of the way in which pure biologists resort to chemical language to explain their conceptions of this problem.

These two examples serve to illustrate how closely the great biological problems are bound up in the chemistry of the cell proteins and suggest fields for our activity. It will therefore be evident, I think, that all biologists are concerned in further extensions of our knowledge along the following important lines: first, the nature of the protein molecule; second, the condition of proteins in the cell; third, the nature of protein compounds. I have therefore divided my discussion under these heads with the view of summarizing our present knowledge.

The nature of the protein molecule. The work of investigators culminating in the now classical work of Fischer on the polypeptids, has shown that proteins are essentially alike. We classify them on the basis of minor differences rather than on any molecular structure. Taylor has carried the synthetic processes still further in his investigation of the manner in which amino acid cleavage products may, by the reversible action of trypsin, be carried to the synthesis of a compound of undoubted protein nature and essentially like protamin.

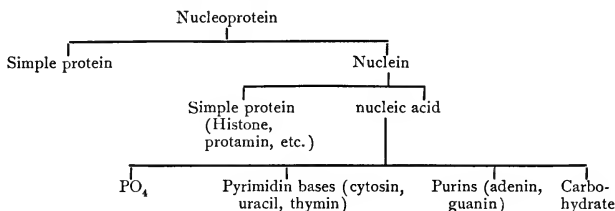
We now can see a reason for the amphoteric character of the protein molecule in the presence of side chains of basic (NH_2) and acidic (COOH) natures. The proportions of these determines

the predominance of basicity in histones, neutrality in albumins, and acidity in nucleoproteins and mucoids. Robertson has pointed out how the presence of these two types of radicals in the molecule explains the tendency to polymerisation and the production of "internal salts." If we represent the compound as



it is easy to see that these radicals may unite with acid and basic groups to form addition compounds. On the other hand the NH_2 radical unites, in many cases, with the COOH group within the compound. The extent to which this can take place gives us new views of the possible complexity of a protein molecule. Ostwald has gone so far as to suggest that a protein solution has a structure, is in fact a single giant molecule and that all its parts are chemically combined chains.

The study of the various nucleoproteins by Kossel, Osborne, Levene, and others, has also thrown light upon the way in which protein molecules may be constructed. The following scheme shows the main cleavage lines for nucleoproteins:



Much work has been done to establish the identity of the nucleic acids of plant and animal cells with one another and the present conclusions all point to such an identity. Work has also been done to establish the nature and intramolecular connections of the carbohydrate radicals. In a recent paper Levene has suggested that in the nucleic acid of the liver and pancreas we have a pentose which he calls *d*-ribose, and that this nucleus is bound to the PO_4 by its OH group and to the purins by its aldehyde group. It is generally agreed that the carbohydrate portion of a nucleoprotein is pentose-yielding though it may, in a few cases, yield hexose.

Investigation of the pyrimidins as a possible method of distinction has led Levene to conclude that all animal nucleic acids contain three such bases, cytosin, thymine, and uracil, while plant nucleic acids contain usually only two, uracil and cytosin.

An interesting suggestion by Robertson shows how the chemical study of the protein molecule indicates possible solutions of physiological problems. In cell division the problem is to account for separation of the nuclei. It is evident that during this process nuclein is being formed rapidly. Lecithin is a probable source of the PO_4 for this material. Loss of this PO_4 may result in the transformation of the cholin residue into a cholin soap. This soap would lower the surface tension in the equatorial plane and hence account for the separation of the nuclear parts. The proposition is, of course more suggestive than conclusive.

In spite of extensive investigation of the cleavage products the problem of the actual configuration of any typical protein molecule is still unsolved. A factor that makes the solution still more difficult is the tendency of proteins to alter. A classical method for preparation often results in an entirely unexpected product. In recent preparations of histone in this laboratory I have encountered some surprising anomalies in this respect. It must also be stated that we are not even sure that our extracted products preexisted in the cell plasma. As a result of extraction procedure they may become as different as coagulated albumin is from soluble albumin.

Condition of proteins in the cell. The last statement suggests to us the importance of knowing more of the physical state of cell proteins. It has long been a question whether colloidal solutions exert any osmotic pressure. While the question is still unsolved evidence seems to answer the question in the affirmative, although such pressure is at best relatively slight. Hardy showed that colloidal particles are electrically active. The question then arose as to whether this was a function of the particles themselves or due to impurities. The use of osmometers permeable to salts tended to clear up this point and with certain protein solutions osmotic pressure was definitely shown to exist when such osmometers were used. Lillie showed, however, that this pressure was not simply a function of concentration and temperature but that

electrolytes influenced the pressure to a remarkable extent. His work has been confirmed by others. While granting therefore, a degree of osmotic pressure to colloids, the influence of other factors is so great as to show the futility of trying to calculate molecular weights by this method. There is also still much controversy as to how electrolytes influence osmotic pressure. Robertson claims that proteins exist as ion proteins; that the contained salts are not impurities but rather parts of the normal protein molecule. In that event purification may be actually cleavage and may carry us too far.

The ultramicroscope has thrown some light on the question of whether protein solutions are suspensions or solutions. With it we can detect particles of 4×10^{-6} mm. diameter or ten times the estimated size of ordinary chemical molecules. The great majority of protein solutions show, with this instrument, visible particles, tending to confirm the idea of suspension. It is not certain, however, that these are not molecules. Lindner and Picton have shown by the study of colloidal solutions of arsenious sulfide that there is no hard and fast line to be drawn between colloidal solutions and crystalloidal solutions. They find that arsenious sulfide solutions may exist in four states:

1. Aggregations visible under the ultramicroscope.
2. Free from visible particles, but indiffusible.
3. Containing invisible particles which diffuse but are retained by a porous cup.
4. Containing invisible particles which diffuse, pass through a porous cup but scatter the light.

We have here a substance of relatively simple chemical character exhibiting every characteristic of suspension, colloidal solution and crystalloidal solution.

Investigation of the Brownian movement has shown that it is probably merely a phase of molecular energy slowed down by the size of the aggregate.

In short we do not know whether to regard a given protein solution as a true solution of large molecules, or as a suspension; as contaminated by salts, or as holding them wholly in molecular combination. We are, however, justified in looking forward to

the time when the relation between colloid and crystalloid may be viewed as merely a matter of size of molecules. Adsorption is a new term to meet this condition of salt and protein combination and may clear up some of our problems by showing the significance of adsorptive affinity as distinct from ordinary chemical affinity.

Compounds of proteins. Many so-called protein salts have been made in the laboratory. Many have been produced in our own laboratory. They show properties which would lead us to consider them ordinary chemical compounds, in some cases, and adsorption products in others. Dyes, too, seem to enter into definite chemical combination with proteins.

In general, then, I have tried to show that on all the points of special interest as regards cell proteins the state of our knowledge is extremely meagre, but that already much has been done and that there is great opportunity for the biochemical study of proteins to aid materially every other branch of biology. That it already has helped is shown in its contributions to the study of problems like fertilization, dietetics, protein metabolism and its relation to disease.

VI. INTRACELLULAR EXTRACTIVES

Isidor Greenwald

The term "extractives" is generally applied for convenience to all the organic constituents of cells and tissues which occur in minute proportions and which cannot be classified under any of the other subdivisions of to-night's program. Included among the extractives are substances of the greatest diversity. Some of them, such as urea, are end-products of metabolism, while others, such as creatin, may be of great functional significance in cells.

Because the extractives occur only in small proportions, investigation of their significance is very difficult. Of all the sources of supply, the commercial extract of beef is the most convenient. Consequently the extractives obtained from it have been studied most carefully. Then too, muscle contains more extractive substance than does any other tissue. Moreover, the extractives of other tissues are qualitatively similar to those of muscle, though quantitatively much difference exists. For this reason these remarks are confined almost entirely to the muscle extractives.

Among the muscle extractives are a number of *basic substances* which contain the trimethyl group. Their small quantity indicates that they are not primary cell constituents but decomposition products derived from lecithans.

Inosite is widely distributed in the animal organism, but muscle, particularly cardiac muscle, is richer in it than is any other tissue. Proof of the utilization of inosite in the animal body is still lacking. When fed to rabbits none can be recovered from the urine. This may be due to complete catabolism or to simple deposit in the tissues. The latter supposition is strengthened by the occurrence of inosite in the urine whenever the volume is much increased. When injected subcutaneously, a large part of it is excreted in the urine, probably owing to its appearance in the circulation in abnormal concentration. That inosite is catabolized is indicated by the results of Mayer¹ who found considerable amounts of lactic acid in the urine of rabbits to which he gave inosite subcutaneously. He was, however, unable to obtain evidence of glycogen formation from inosite. These results are rather contradictory, for Mandel and Lusk² have shown that glucose can be formed from lactic acid and it is difficult to understand why glycogen should not have been formed from the inosite by way of lactic acid and glucose.

Much importance was ascribed to *nucleon*, or phosphocarnic acid, by Siegfried, but more recent investigations indicate that it is not a chemical compound but only a mixture of several products obtainable from meat.

The free *hypoxanthin* of muscle has been the subject of considerable study but as yet we know little of its significance. The work of Voegtlin and Jones³ has shown quite conclusively that muscle tissue does not contain adenase and that consequently adenin is probably not the precursor of the hypoxanthin of muscle. Burian⁴ showed that muscular work increased the excretion of uric acid and purin bases in the urine; also the amount of these substances in the liquid used to perfuse isolated muscles.

Two precursors of hypoxanthin suggest themselves, *carnin* and

¹ Mayer: *Biochemische Zeitschrift*, 1907, ii, p. 393; 1908, ix, p. 533.

² Mandel and Lusk: *American Journal of Physiology*, 1906, xvi, p. 129.

³ Voegtlin and Jones: *Zeitschrift für physiologische Chemie*, 1910, lxxvi, p. 250.

⁴ Burian: *Zeitschrift für physiologische Chemie*, 1905, xliii, p. 532.

inosinic acid. Of the former we can say little except that it yields hypoxanthin on oxidation. Inosinic acid has been known since the time of Liebig, who first isolated it. Since that time it has received considerable attention. Chemically it is similar to, though much simpler in constitution than, the nucleic acids in other tissues. It differs from these in containing an oxypurin instead of an aminopurin radical. The work of Burian, and of Levene and Jacobs,⁵ indicates that hypoxanthin is in glucoside-like combination with *d*-ribose. This in turn is combined with orthophosphoric acid to form an ester. Other nucleic acids probably have a similar constitution but contain instead of ortho-phosphoric acid one of the complex poly-basic phosphoric acids. These complex acids are united with a pentose or hexose, which in turn is combined with purin and pyrimidin bases. That inosinic acid plays a somewhat different role in muscle than do the nucleic acids of other tissues is indicated by the comparatively large amount in which it occurs. This is much greater than would be expected from the small number of nuclei in muscle.

Carnosin, which was first obtained from extract of beef by Gulewitsch and Amiradzibi,⁶ is a substance about whose nature and significance little is known. Histidin has been isolated from the products of hydrolysis of carnosin and it has been suggested that carnosin is a dipeptid of histidin and alanin. Occurring as it does in fairly large quantity, von Fürth and Schwartz⁷ having found almost as much nitrogen in the form of carnosin as they did in the form of creatin, it is probably of considerable physiological importance.

The extractive that has been studied more than any other is *creatin*. This is apparently an essential constituent of muscle. It is probably the precursor of the creatinin of the urine but the relation between the two is not as simple as was formerly believed to be the case. Folin⁸ has shown that the conversion of one into

⁵For a general review of the biochemistry of nucleic acids, see Levene: Journal of the American Chemical Society, 1910, xxxii, p. 231.

⁶Gulewitsch and Amiradzibi: Zeitschrift für physiologische Chemie, 1900, xxx, p. 565.

⁷Von Fürth and Schwartz: Biochemische Zeitschrift, 1911, xxx, p. 413.

⁸Folin: The Chemistry and Biochemistry of Kreatin and Kreatinin. Festschrift für Olof Hammarsten, 1906 (III).

the other is by no means as readily accomplished as had been assumed. Folin and others have found that the ingestion of creatin does not increase the creatinin of the urine. If given in doses that are not extremely large, especially when the subject is on a low protein diet, all the nitrogen of the creatin may be retained. With larger amounts, or in conjunction with a high protein diet, creatin appears in the urine.

Muscular work, if the food be sufficient, is quite without effect upon the *creatinin* excretion. The work of van Hoogenhuyze and Verploegh⁹ and Shaffer¹⁰ is quite conclusive in this regard. Brown and Cathcart,¹¹ however, have found that while the total amount of creatinin in isolated frog muscle was increased by work, in mammalian muscle, with circulation intact, it was decreased. Considerable doubt has been thrown upon their results by Pekelharing and van Hoogenhuyze,¹² who found that work did not affect the creatin and creatinin contents of frog muscle.

Although not influenced by the amount of work done, the creatinin excretion bears a direct relation to the general state of the muscular tissues. Conditions that affect ability of the muscles to do work are characterized by a low creatinin excretion and sometimes by the appearance of creatin in the urine.

The interesting work of Pekelharing and van Hoogenhuyze should be mentioned in this connection. These observers found that, although the tetanizing of muscle did not increase the creatin content, conditions which increased the tone of the muscle were characterized by a high content of creatin. They are of the opinion that creatin is the product of a specific tone-metabolism, which is very different from the ordinary contraction metabolism. Lingle,¹³ from histological studies upon smooth muscles, has come to a contrary conclusion. He believes that tone is a condition of partial contraction.

⁹ Van Hoogenhuyze and Verploegh: *Zeitschrift für physiologische Chemie*, 1908, lvi, p. 415.

¹⁰ Shaffer: *American Journal of Physiology*, 1908, xxii, p. 445.

¹¹ Brown and Cathcart: *Biochemical Journal*, 1909, iv, p. 420.

¹² Pekelharing and Van Hoogenhuyze: *Zeitschrift für physiologische Chemie*, 1910, lxiv, p. 262.

¹³ Lingle: *American Journal of Physiology*, 1910, xxvi, p. 361.

No precursor of creatin is known. It is possible that it is derived from arginin which is chemically closely related to it, but no evidence of such change has been found.

Von Fürth and Schwartz⁷ recently found that cardiac muscle of the horse contains much less creatin than does its skeletal muscle, but rather more purins and carnosin. They also noted that the amount and character of the extractive nitrogen was the same in normal and in fatigued dog muscles. Apparently then the extractives are not involved or affected in the ordinary contraction metabolism. They are, however, intimately connected with the life of the muscle cells.

VII. INTRACELLULAR ENZYMES

Alfred P. Lothrop

Enzymes are colloidal in nature and do not dialyse through parchment paper or do so with extreme slowness. It has been claimed by some that in solution they are electrically charged. Being colloids they tend to carry down with them, by adsorption, constituents of the solutions from which they are precipitated. It is very difficult to obtain pure enzymes unassociated with other substances. Whether the enzymes are proteins or not is still uncertain. Some of the purest preparations have been found not to give the characteristic protein reactions but the reactions may have been so faint as to be overlooked. In very recent work on invertase¹ it was found that as long as the enzyme remained active it gave the biuret, Millon and xanthoproteic reactions, suggesting that the essential part of the ferment is a protein. Very pure preparations of pancreatic amylase were found by Sherman and Schlesinger to give pronounced xanthoproteic, tryptophan, biuret and Millon tests. Water solutions coagulated on boiling but the filtrates gave a rose pink reaction in the biuret test.² Some of the enzymes are secreted in an inactive form or zymogen and are activated by a co-enzyme or kinase. Their action is markedly influenced by temperature and other conditions, the reaction of the liquid being especially important biologically. The products of their own

¹ Matthews and Glenn: *Journal of Biological Chemistry*, 1911, ix, p. 29.

² Sherman and Schlesinger: *Journal of the American Chemical Society*, 1911, xxxiii, p. 1195.

action exercise a retarding influence. Some substances which destroy the life of protoplasm have no material effect on the power of enzymes showing that enzyme action is distinct from protoplasmic activity.

Enzymes may be divided into two classes, intracellular and extracellular. The extracellular enzymes act outside the cell in which they are formed or after being transformed into active enzymes from their zymogens; they may be called secretion enzymes, *e. g.* ptyalin, pepsin and trypsin. The intracellular enzymes, on the other hand, exert their action within the cell and can as a rule be extracted only with great difficulty. They are fixed, and even after the death of the cell are set free only very gradually if the tissue is kept intact.

The view has been expressed "that enzymes are not chemical individuals but that various kinds of bodies may have conferred upon them properties which cause them to behave like enzymes, so that we have to deal with properties rather than substances." Still another theorist has proposed the idea that enzymes act as radioactive bodies and that radiations are the cause of the chemical activity of enzymes.

There are very many different kinds of intracellular enzymes. Practically every group of enzymes has several intracellular representatives. In fact enzymes are cellular in origin, a great majority of the enzymes exercise their activity within cells, and most of the essential intracellular chemical changes are the results of activities in which enzymes are involved.

Various methods have been employed for the isolation of intracellular enzymes, such as autolysis, mincing the tissues, grinding with quartz sand and filtration under high pressure. These enzymes can be extracted with saline solution saturated with chloroform or ether.

The unit of living substance may be considered as consisting of a nucleus with which numerous side chains are connected, in most cases of a protein-like nature. These side chains are bound up in different ways and with different degrees of firmness. With kidney it has been shown that by changing the salinity of the perfusing fluid from 1 per cent. to 4 per cent. the disruption was

increased six-fold, the increase being the same for both the protein and the erepsin. On the other hand if already perfused saline is sent through a second or a third time, the protein disruption is diminished to one seventh, while that of the enzyme is increased twenty fold. Different enzymes are apparently bound with different degrees of firmness, for in two hours dilute alcohol will remove 55 per cent. of the total diastase and only 14 per cent. of the trypsinogen from minced pancreas.

The intracellular enzymes appear to be bound up in somewhat the same way as the side chain receptors of Ehrlich. They are bound with sufficient firmness to prevent their being cast off into the blood stream in other than small quantities, some, such as maltase, being liberated in larger amounts. The secretion enzymes, on the other hand, are liberated in large quantities whenever needed, their linkage being more readily snapped by chemical or nervous stimulus. A few of the intracellular enzymes can be extracted with comparative ease; glycerin apparently extracts endo-erepsin as readily as exotrypsinogen and amylopsin.

Enzymes may be regarded as similar to Ehrlich's "receptors of the second order." Each enzyme has a group that may be called its haptophoric group, which is supposed to attach itself to a particle of substrate and act upon it by its so-called zymophore group. The intracellular enzymes are directly comparable to agglutinating receptors while the secretion enzymes are comparable to receptors which are over regenerated and cast off into the blood stream, as when the blood of one animal is injected into that of another.

In some cases enzymes apparently act as "receptors of the third order," though there is no direct proof of this. They do not bind themselves directly to particles of substrate but only through the intervention of a third substance or amboceptor, as for example in the conversion of fibrinogen into fibrin. Again it has been shown that ptyalin, inactivated by heating to 53° C. can be reactivated by adding blood, showing probable existence in ptyalin of a thermostable substance and a non-specific kinase present in the blood and tissue extracts as well as in saliva.

There is other evidence of the presence of haptophorous and

zymophorous groups in the enzyme molecule. Rennin solution filtered through a Berkefeld filter loses its milk-curdling power to a greater extent than its power to neutralize anti-rennin. The solution seems to contain enzyme molecules with both groups, and molecules with the haptophore group alone. The pores of the filter apparently retain a larger proportion of the former than of the latter.

Enzymes closely resemble toxins in that they stimulate the tissues to form anti-bodies; anti-rennin, anti-pepsin, and trypsin, etc. It seems probable that every enzyme injected under proper conditions would induce the formation of a specific anti-body. This is an argument in support of the protein-like nature of the enzymes, although there is also evidence against this view.

Investigations on invertase and diastase indicate that enzymes are made up of two parts, a combination of a colloid (a mannose-gum in the case of invertase) with an active principle (protein in nature). The colloid is generally related chemically to the substance upon which the enzyme acts; for example, mannose-gum in invertase and arabanose gum in diastase. So long as the enzyme is combined with its carrier it remains inactive but is broken away by slight changes in reaction. In the cells the various enzymes may thus be anchored and rendered inert in combination with colloids. They may accumulate in this inert form, from which slight local changes in reaction set them free to produce their characteristic action locally.³

Many or most of the chemical processes occurring in living cells are dependent on the action of intracellular enzymes, directly or indirectly, and naturally their great importance has attracted very many workers to this field of research. As yet very little is definitely known regarding their chemical structure and of the way in which they are combined in the cell in spite of the vast amount of work that has been done upon them. When these two points have been definitely established a great many of the life processes will be understood which are now extremely obscure.

³ Matthews and Glenn: *Loc. cit.*

VIII. FACTORS IN IMMUNITY

Reuben Ottenberg

Under the general heading of "immunity" is now classed a rapidly growing and somewhat heterogeneous volume of knowledge, much of which has nothing to do with immunity to disease and really belongs to the general subjects of metabolism or ferment action. There is, for instance, little doubt that two of the immunity reactions which most immediately affect cells—cytolysis and agglutination—only in rare cases have any function in real immunity. The true explanation of these phenomena is still to be found, but they are probably connected in some way with metabolism of the cell.

The study of immunity commenced as a branch of bacteriology. Ehrlich early saw the chemical nature of the processes involved and his side chain theory which forms the basis of most recent thought on the subject is essentially a chemical conception. Nevertheless there is as yet extremely little knowledge of the actual chemical processes involved in any immunity phenomenon. This is due not only to the highly complex nature of the phenomena but to our inadequate knowledge of the exact chemical composition of the proteins and lipins. The highly specific nature of immunity reactions (which in many cases offer by far the most delicate tests for certain specific substances) indicates that only an exact chemical knowledge can ever offer a complete explanation. As yet we are still trying to find out the general nature of the processes involved.

To review the innumerable ways in which the conceptions of immunity have affected our views of the life processes in cells would be a very large task. I prefer to take one typical reaction involving cells and show the way in which chemical conceptions have entered into our explanation of it.

Bordet has pointed out that the phenomena of agglutination are due to changes of surface tension between cells and suspending fluid. An experiment of Lauder Brunton's illustrates this point. When little pieces of wood (matches) smeared with soap are put in water they float about with no especial tendency to come to-

gether. If the reaction of the fluid is made acid the matches are seen to clump together in a way which is strikingly suggestive of the agglutination of bacteria. When the reaction is neutral the soap is soluble and there is no sharp surface of demarcation between solid and fluid. When the liquid is acid, however, an insoluble film forms at once and constitutes a new surface, the tension of which induces the aggregation of the floating masses. (These phenomena were demonstrated.)

Numerous experiments have shown that serum agglutination whether natural or due to immunization, is caused by a reaction between two substances, of which the one, agglutinin, is in the serum and the other, the agglutinable substance (or agglutinum), is in the cell. In producing its effect the agglutinin disappears from solution in the serum (is "absorbed" by the cells). It appears that the reaction which occurs between the two substances results in the formation of an insoluble product at the surface of the cell.

That this is the case seems probable, in the first place, from the fact that under certain conditions the agglutinable substance can be extracted from the cells, and treated with agglutinative serum. When this is done, a precipitate occurs. This was first observed by Kraus who noticed that in old broth cultures of typhoid bacilli, after removal of the bacilli, a precipitate could be formed by addition of typhoid-agglutinative serum. Klein has made similar observations with red blood cells and hemagglutinative serum. That the process is in the nature of a precipitation at the surface of the cell is indicated also by the experiment of Moreschi. He showed that red blood cells treated with such a dose of agglutinating serum as in itself is entirely insufficient to agglutinate, undergo extraordinarily rapid and complete agglutination, if there is added a small amount of serum containing precipitin for the agglutinative serum used.

The chemical nature of the two reacting substances is unknown. The highly specific nature (within certain limits) of agglutination phenomena indicates that the substances must be extremely complex. The part they play in intracellular life is still obscure. That they are functionally important in cells is a reasonable assumption.

THE MEETING OF THE SECTION OF BIOLOGICAL
CHEMISTRY OF THE AMERICAN CHEMICAL
SOCIETY, AT INDIANAPOLIS, JUNE
28 TO JULY 1, 1911

PROCEEDINGS REPORTED BY THE CHAIRMAN,

CARL L. ALSBERG

I. ON THE ORGANIZATION OF A DIVISION OF BIOLOGICAL
CHEMISTRY OF THE AMERICAN CHEMICAL SOCIETY

For some years there have been occasional meetings of the Biological Section of the American Chemical Society. Professor Bancroft, President of the Society in 1910-11, decided to attempt to put the Section on a firmer basis with a view eventually to organizing it into a Division. The writer was asked to take the chairmanship of the section for the meeting in Minneapolis, December, 1910.

The question arose whether there was a need for such a section, since the American Society of Biological Chemists, a very successful organization, was already in the field. That a section would serve any useful purpose seemed open to doubt. Indeed it seemed possible that a biological section in the American Chemical Society would do more harm than good, by weakening the independent society. However, mature deliberation led to the conviction that such a section might well serve a very useful purpose without in the least competing with or injuring the independent Society. The members of the Society of Biological Chemists are chiefly medical men, or at least, men who hold positions on medical faculties. The natural result is that the main interest of the Society is along the lines of mammalian physiology. This, however, is but one of the fields of biochemistry. The chemistry and chemical physiology of plants, fungi, bacteria, and certain pure science fields of agriculture, fermentation chemistry, and the like, are not at present receiving the attention they deserve. Nevertheless there is an increasing number

of workers in these fields. It seemed worth while to make the attempt to bring these men together in a section and to give them a forum. Botany in this country at the present time is in the same stage in its relation to biochemistry that medicine was a decade or more ago. The phytochemist, and even the plant physiologist, have no place where they may present their work before any but an audience of taxonomists and morphologists. They are widely scattered, have no place where they may come in touch with one another, and are hardly recognized as legitimate workers. But few chairs on these subjects exist. It seemed possible that if these scattered workers were brought together in a section the result might be to stimulate research along these lines and to perform missionary work in the advancement of this field.

The young man working alone in this field in a smaller or outlying institution finds it difficult to get in touch with the older, more experienced men. It is the young man, however, who needs societies and who derives the most benefit from their meetings. The American Chemical Society is a perfectly democratic organization. Election to membership is very easily attained. Consequently its Biological Section would be open to isolated workers and thus might serve a very useful purpose as a training ground.

Finally, biochemistry has become a legitimate field of chemistry. A great society like the American Chemical Society endeavors to cover the entire field of chemistry. It would certainly be very injurious to the progress of biochemistry on this continent if our most important chemical society ignored it.

It was therefore decided to reorganize the Biological Section of the American Chemical Society along these lines. The result has been very gratifying. Two meetings have been held, one at Minneapolis and one at Indianapolis. The programs have been large and interesting. The attendance has been good, and the discussion spirited. The result has been that at the Indianapolis meeting the Section voted to request the Council of the American Chemical Society to organize the Section into a Division. This request will be acted upon at the Christmas meeting of the Society in Washington. The object of forming a Division is to give the Section permanence. A section exists at the option of the general

officers of the Society. A division is self-perpetuating, electing its own officers, etc. In most other executive respects it does not differ from a section. It is hoped that by the organization of this Division, the non-medical fields of biochemistry may also be advanced.

II. ABSTRACTS OF THE COMMUNICATIONS AT THE INDIANAPOLIS MEETING OF THE SECTION OF BIOLOGICAL CHEMISTRY OF THE AMERICAN CHEMICAL SOCIETY

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The Effect of the Club Root Disease upon the Ash Constituents of the Cabbage Root

HOWARD S. REED

(Laboratory of Plant Pathology, Virginia Agricultural Experiment Station, Blacksburg, Va.)

Ash analysis of healthy and diseased cabbage roots reveals appreciable variations in the amounts of certain constituents while others vary but slightly. In the diseased roots there was an appreciable increase in the amounts of calcium, magnesium, phosphoric acid, potassium and sulphuric acid, *i. e.*, an increase in the amount of "essential" elements.

The greatest increase of any single constituent was in the case of potassium. The increase of potassium appears to be coupled with an increase of protoplasmic substance and accumulation of starch.

The proportion of calcium to magnesium is greater in the diseased roots. The same is also true of the proportion of potassium to sodium, but there is no material difference in the proportion

of magnesium to phosphorus. The differences in the amounts and proportion of ash constituents appear sufficiently well marked to indicate a more or less definite correlation in the metabolism both of healthy and of diseased plants. (*Phytopathology*, 1911, i, p. 159.)

The Effect of Frost on the Aromatic Constituents of the Peppermint Plant

FRANK RABAK

(Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations, Bureau of Plant Industry, Department of Agriculture.)

The oil from plants (*Mentha piperita*, L.) frozen November 26, was compared with the oil distilled earlier in the same season from plants in the same stage of growth (12–18 inches in height without buds and consisting largely of leaves). Some of the differences reported are given in the following table:

	Oil from frozen plants,	Oil from normal plants,
Specific rotation	—25.2°	—18.4°
Readily soluble in 80 per cent. alcohol in excess.		Turbid in 1½ vols. or more.
Acid number	0.9	0.8
Ester number	97.4	25.2
Acetylation number	206.	126.
Menthyl Acetate	34.3 per cent.	8.8 per cent.
Free Menthol	40.7 per cent.	31.8 per cent.

The chief effect of frost was therefore to increase the esters and also to a certain extent, the free menthol. It has been suggested that esterification is the result of a reversible enzyme reaction checked by water, since any condition which accentuates the elimination of water favors esterification in the plant. The author suggests that the mechanism of the action of freezing may be to check the absorption of water and possibly to render useless the water circulating in the plant. Esterification by aid of an enzyme with a catalytic action may have continued, the water formed being removed by freezing and thus mitigating the chance of a reverse reaction.

Menthol has been shown to be capable of oxidation to menthone. Since free menthol was slightly in excess in the oil of the frozen plant, perhaps another effect of freezing is to lessen oxidation in the plant tissues.

Absorption and Excretion of Salts by Roots, as Influenced by Concentration and Composition of Culture Solutions

I. CONCENTRATION RELATIONS OF DILUTE SOLUTIONS OF CALCIUM AND MAGNESIUM NITRATES TO PEA ROOTS

R. H. TRUE AND H. H. BARTLETT

(Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations, Bureau of Plant Industry, Department of Agriculture.)

Secondary roots of the pea will not develop in $m/5000$ $Mg(NO_3)_2$. A calcium to magnesium ratio of $1/10$ is the lowest ratio at which calcium entirely inhibits the harmful effect of magnesium in solutions of this concentration. In solutions of such concentration that magnesium alone would not prevent the formation of secondary roots (*e. g.*, $m/20,000$) the calcium to magnesium ratio which brings about perfect root development is nearer $1/100$. In solutions of whatever concentration the calcium to magnesium ratio most favorable to absorption of salts is $1/1$.

For calcium nitrate solution used as a culture medium, or for magnesium nitrate solution, or for mixtures of the two in any ratio, there is a definite concentration above which roots absorb more electrolytes than they excrete and below which they excrete more than they absorb. This equilibrium concentration is $12 m/500,000$ for $Mg(NO_3)_2$ and $7 m/50,000$ for $Ca(NO_3)_2$. For solutions of the two in the lowest ratio which permits perfect root development ($Ca:Mg:1:10$) the equilibrium concentration is $8 m/500,000$, almost as low as for $Ca(NO_3)_2$ alone. For solutions mixed in the most favorable ratio for absorption (*i. e.*, $5/5$) the equilibrium concentration is $5 m/500,000$, lower than for $Ca(NO_3)_2$ alone.

Creatinine in Plants and in the Medium in Which They Grow

M. X. SULLIVAN

(*From the Laboratory of Fertility Investigations, Bureau of Soils.*)

By means of the creatinine zinc chloride method, creatinine was found in the *alcohol* extracts of wheat seed, wheat seedlings, wheat bran, rye, clover, alfalfa, cowpeas and potatoes, and in *water* extracts of wheat and tea. It was found also in the water in which seedlings had grown and in the water and glycerine extracts of planted soil. Though the amount of creatinine and its congener creatine in vegetable matter is small, it is worthy of attention since by the decay of plants, by direct cell sloughing or even by osmosis, the creatinine and creatine are left in the water and soil.

The Effect of Temperature on the Respiration of Fruits

H. C. GORE

(*Bureau of Chemistry, U. S. Department of Agriculture.*)

This paper presents the results of an exact study of the respiration of fruits as determined in specially designed absorption and constant temperature apparatus. Many fruits of several different types were investigated in this manner and great variations in respiratory intensity were found. However, when the results were plotted in curves these variations disappeared and the curves were of the same general type. Small fruits and those tending to mature early respire with considerable rapidity while slowly growing and maturing fruits like those of the citrus type respire very slowly. The common ones such as apples, grapes, and peaches formed a group characterized by a respiration of moderate intensity. Mathematical expressions were deduced for the respiration values under different conditions. The rate of respiration more than doubled for each 10° rise in temperature. Field experiments showed that picking had very little effect upon the respiratory function. (Bulletin 142.)

The Phosphorus Assimilation of *Aspergillus Niger*

ARTHUR W. DOX

(From the Chemical Section of the Iowa Agricultural Experiment Station.)

The necessity for some form of phosphorus in culture media for lower fungi has long been recognized. Notwithstanding the variety of phosphorus compounds occurring in nature, very few have been tested with regard to their availability as sources of this element for mold cultures. Among the substances tested in this experiment were phytin, sodium glycerophosphate, sodium nucleinate, lecithin, casein, ovovitellin, ortho-, pyro- and metaphosphates, hypophosphites and phosphites. All but the last two, which contain trivalent phosphorus, were readily utilized.

Fermentation and Putrefaction

ARTHUR J. KENDALL

(Department of Preventive Medicine and Hygiene, Harvard Medical School.)

As shown by the work of the author and others, utilizable carbohydrates protect nitrogen from attack by bacteria. This finds its analogue in the metabolism of higher forms. Fermentation takes precedence over putrefaction. For the purposes of this paper, by fermentation is meant the action of bacteria upon carbohydrates; while by putrefaction is meant the action of bacteria upon nitrogenous substances. The two phenomena, fermentation and putrefaction, are antagonistic processes: the obligate putrefactive bacteria cannot, as a rule, grow in media in which active fermentation is going on, because the acids produced inhibit their development. There is a third group, the facultative organisms, which are able to adapt themselves to both kinds of food. This is an important new conception. Thus, in the presence of dextrose the diphtheria bacillus elaborates no toxin, while in its absence large amounts are

formed. *B. coli* behaves similarly. Not only do the products vary, but the composition of the bacteria themselves may be altered. All these considerations will prove of great importance in practice.

The Carbon-Nitrogen Ratio in the Decay of Protein Compounds

JACOB G. LIPMAN AND H. C. MCLEAN

(*New Jersey Experiment Station, New Brunswick, N. J.*)

In processes of decay the oxidation of the carbon and nitrogen in protein compounds is influenced by the physical and chemical make-up of the decaying substances, the prevailing moisture and temperature conditions, and the prevailing micro-organic flora. Some of these factors were studied by the authors by means of a method especially devised for the purpose.

Weighed quantities of soil, containing definite amounts of dried blood, tankage, cottonseed meal, etc., were placed in covered tumblers and kept under bell-jars of known capacity. Under each bell-jar there was also placed a measured quantity of standard barium hydrate. The latter was renewed daily, for a period of eight to ten days, and the amount of carbon dioxid absorbed was determined by titration against $n/10$ hydrochloric acid. Similar quantities of soil without additions of nitrogenous materials were employed as checks. It was possible, thus, to estimate the amount of carbon dioxid formed from the protein substances in any given length of time. The daily production of ammonia in a parallel series was determined by transferring the contents of the tumblers to copper flasks, adding water and calcined magnesia, distilling off and titrating the ammonia formed.

The determination of the carbon dioxid on the one hand, and of the ammonia on the other, furnished accurate data on the relative rate of oxidation of the carbon and the nitrogen in the nitrogenous materials employed. It was shown in this manner that the presence of large amounts of readily decomposable non-nitrogenous organic matter lessened the amount of ammonia found. On the other hand, the amount of carbon dioxid produced was increased

to a marked degree, thanks to the presence of the non-nitrogenous compounds. It was thus demonstrated that in the decomposition of protein bodies by microorganisms the amino compounds and ammonia formed were utilized for the formation of new bacterial cells, and such was the intensity of their development that most of the ammonia was again changed into complex, insoluble substances. Similar studies were made on materials containing various proportions of nitrogenous and non-nitrogenous compounds. It was shown that in decay processes in the soil the carbon-nitrogen ratio of the oxidized products was profoundly modified by the carbon-nitrogen ratio of the initial substance, as well as by the proportion of moisture present.

Biochemical and Toxicological Studies upon *Penicillium*

C. L. ALSBERG AND O. F. BLACK

(*Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations, Bureau of Plant Industry, Department of Agriculture.*)

Under certain conditions the green mould *Penicillium puberulum* Bainier, produces a new complex organic acid. It is moderately toxic and has been named penicillic acid.

The Optical Forms of Lactic Acid Produced by Pure Cultures of *Bacillus bulgaricus*

JAMES N. CURRIE

(*Laboratory of Agricultural Chemistry, University of Wisconsin, Madison, Wis.*)

Pure cultures of *B. bulgaricus* were isolated from various sources, *i. e.*, cow's milk, Cheddar cheese, human saliva, human feces, cow feces, horse feces, brewers' malt and kraut. The optical form of the lactic acid produced in sterile milk was studied by means of the water content of the zinc lactate. The majority of these cultures produced pure dextro-lactic acid; some produced a mixture of

unequal quantities of dextro- and levo acid; others produced a mixture of *equal* quantities of dextro- and levo-acid, or pure inactive acid; and one culture produced pure levo acid. The results suggest the possibility of differentiating bacteria by this method, when cultural characteristics and morphology furnish no certain marks of distinction.

Nucleic Acids in Soils

EDMUND C. SHOREY

(Laboratory of Fertility Investigations, Bureau of Soils.)

From several soils, by extraction with dilute sodium hydroxide, an amorphous light colored body has been obtained having the general properties of nucleic acids.

This body on heating with hydrochloric acid gives decomposition products among which phosphoric acid, pentose sugars, cytosine, and a mixture of xanthine bases have been identified. Reactions indicating the presence of levulinic acid have also been obtained after hydrolysis with a mineral acid.

In the mixture of xanthine bases the presence of hypoxanthine has been definitely determined. The body itself has the general properties as to solubility and precipitation common to nucleic acids.

Conditions for Tannic Acid Fermentation

LEWIS KNUDSON

(Department of Botany, Cornell University, Ithaca, N. Y.)

As a result of the fermentation of tannic acid (gallotannic), gallic acid is formed. Van Tieghem first showed that the fermentation of this substance may be effected by the two organisms *Aspergillus niger* and *Penicillium glaucum*. Pottevin and Fernbach simultaneously reported the extraction of the enzyme tannase, the transforming agent. Since that time several other investigators have contributed to the subject.

Experiments made by the writer indicate that if tannic acid

alone is offered as a source of carbon, the gallic acid formed as a result of the tannic acid transformation is utilized in the metabolism of the organism—the greater the growth of the fungus, the greater is the decrease in tannic acid. It is likewise shown that the duration of growth, the presence of other nutrients, and aeration—factors influencing growth mass—were important considerations with respect to the yield of gallic acid.

An infusion of gall nuts contains, in addition to tannic acid and gallic acids, other organic compounds as well as inorganic salts. When cultures are made in which the gall nut infusion is used as the nutrient solution, the tannic acid is transformed; but the gallic acid is not at first utilized. The organism seems to elect the other organic compounds first and then some of the gallic is utilized. There is, then, an election of food by the organism.

If there is offered to *Aspergillus niger* or *Penicillium sp.* in a nutrient salt solution, 10 per cent. cane sugar along with 13 per cent. tannic acid, then the sugar entirely protects the gallic acid formed, from assimilation or use as food by the fungus. A 5 per cent. concentration of sugar is not sufficient to protect the gallic acid, during the growth interval employed.

Experiments were also made in which the fermentation cultures were kept under anaerobic, and also limited oxygen conditions, and the results obtained were compared with those in which growth was permitted under more favorable conditions of aeration and nutrition.

Regulatory Formation of the Enzyme Tannase

LEWIS KNUDSON

(*Department of Botany, Cornell University, Ithaca, N. Y.*)

The work of Fermi, Pfeffer, Katz, Went, Dox and others has shown that the formation of enzymes is influenced markedly by the nutrition of the organism. According to Dox, the production of those enzymes that are not normally developed by the organism cannot be induced in demonstrable quantities by any special nutrition. This statement is not in accord with the results obtained by Went; nor with the more recent work of Harden and Norris work-

ing with yeast, wherein it is shown that there may be induced by special nutrition an enzyme which did not occur normally in the yeast plant. The work of the writer, herewith briefly reported, is also in disagreement with the results of Dox.

The two organisms, *Aspergillus niger* and *Penicillium sp.*, which normally develop on commercial gall nuts when these are moistened and exposed to the air, produce the enzyme tannase; and this enzyme is capable of effecting the transformation of tannic acid into gallic acid and glucose.

Pottevin found that the enzyme tannase was formed in *Aspergillus niger* when it was grown in Raulin's solution in which the sugar was replaced by tannic or gallic acid. The writer has grown the organism in synthetic solutions in which the carbon nutrient, cane sugar, was replaced entirely or supplemented by one of several carbon compounds. In the experiments the effect of each of fourteen different carbon compounds was tested, but the enzyme tannase was produced only when the sugar was replaced by tannic or gallic acid, or supplemented by tannic acid. The gallic acid, furthermore, was not as efficient as the tannic acid in stimulating the formation of the enzyme.

Some work has been done showing that the quantity of a particular enzyme produced irrespective of the character of the carbon nutrient, can be increased in amount by offering the organism the carbon compound which is transformed by the enzyme in question. No work apparently has been reported on the effect of concentration of the transformable substance on the quantity of the corresponding enzyme produced. Employing the two organisms mentioned, the writer made experiments, in which a modified Czapek's solution was the nutrient medium—in this the concentration of sugar was made 10 per cent., and it was supplemented by tannic acid in concentrations varying from 0.01 per cent. to 10 per cent. The quantity of the enzyme produced was augmented by increase in concentration of the tannic acid. None, however, was formed when the concentration of tannic acid was as low as 0.01 per cent.

Similar results were obtained with *Penicillium sp.* *Aspergillus candidus*, *Aspergillus oryzae* and *Penicillium granulatum* cultivated in a synthetic solution in which the carbon was supplied as 5 per

cent. cane sugar and supplemented by 2 per cent. tannic acid also developed the enzyme tannase. *Penicillium expansum* in a similar solution did not develop the enzyme.

The enzyme tannase would fall then in the third class, as described by Went, which class includes only those enzymes which are produced when a particular carbon compound is present in the nutrient solution.

The Synthesis of Fats by the Action of Enzymes

F. L. DUNLAP AND L. O. GILBERT

(*Bureau of Chemistry, U. S. Department of Agriculture,
Washington, D. C.*)

Five grams of oil-free castor bean, 5 gm. of flaxseed, 25.5 gm. of glycerol and 16.7 gm. of Kahlbaum's oleic acid were triturated in a mortar until emulsified. The flaxseed were introduced to perfect the emulsion. It is without action. This emulsion was allowed to stand and its acidity titrated at intervals. After eleven days the loss of acidity was such as to correspond to a disappearance of over 26 per cent. of the total oleic acid present, so that the enzyme of *Ricinus* has undoubted synthetic power.

On the Measurement of the Oxidase Content of Plant Juices

H. H. BUNZEL

(*Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations, Bureau of Plant Industry,
U. S. Department of Agriculture.*)

The author presents a method for the accurate measurement of the physiological processes which are being ascribed to oxidases. The measurements are based on oxygen absorption and are carried out in an apparatus especially devised for the purpose. The experiments are all carried out in an air thermostat maintaining the temperature within a tenth of a degree. The oxidase apparatus

is of such construction that the carbon dioxide formed during the process may be determined separately if desired.

This method for the determination of oxidases in the plant juices has already yielded a number of very interesting results. If pyrogallol is used as the substance to be oxidized, atmospheric oxygen as the oxidizing agent, and potato juice or beet juice as the catalyst, the oxidation proceeds quite rapidly for an hour or two and then reaches a maximum. This maximum has been taken as a measure of the activity of the juice. The maximum quantity of oxygen absorbed with varying volumes of plant juices added proved to be directly proportional to the concentration of the oxidases present. The pyrogallol, oxygen, and oxidase react in purely stoichiometric proportions, *i. e.*, a certain amount of potato juice brings about the oxidation of a definite quantity of pyrogallol by a corresponding weight of oxygen. Excess of any one of these three reacting components is without effect on the end result.

This method has been applied only to potatoes and sugar beets. The oxidase content of healthy sugar beets was compared with that of curly top sugar beets. The leaves of the diseased beets invariably gave higher figures than the normal ones. This remarkable behavior was studied more in detail under field conditions in Utah during the summer and will be reported in one of the publications of the Department of Agriculture.

A Study of the Methane Fermentation in the First Stomach of the Ruminants

SLEETER BULL

(Institute of Animal Nutrition, Pennsylvania State College, State College, Pa.)

Crude fiber or cellulose and starch undergo a fermentation in the paunch of the ruminants with the formation of methane, carbon dioxide, acetic acid, butyric acid and isobutyric acid.

By the artificial fermentation of cellulose, it was found that 1.0 gm. of cellulose yielded 0.033–0.040 gm. of methane.

Omeliansky found that one gm. of cellulose yielded 0.068 gm. of methane, 0.3057 gm. of acetic acid and 0.2038 gm. of butyric and isobutyric acids.

Knowing the energy value of the cellulose—4.220 Cal.—and that of the products of the fermentation, it may be computed that 1.048 Cal. of energy are liberated as “heat of fermentation” in the fermentation of one gram of cellulose. Expressed in terms of methane, 1.1549 Cal. of energy are lost as “heat of fermentation,” for every Cal. of methane excreted by the animal.

Applying this factor to results of experiments upon steers with the respiration calorimeter, in which the amount of methane excreted and the amount of heat emitted after the ingestion of a known amount of food were determined, it is found that in the case of a hay ration, 36.8 per cent. of the “heat of digestion” arose from the methane fermentation. On the hay ration 32 per cent. of the “heat of digestion” arose from the methane fermentation of the carbohydrates.

The Pigmentation of the Adult Periodical Cicada, with a Note on Chemical Anti-Oxidases

ROSS AIKEN GORTNER

(*The Station for Experimental Evolution, Carnegie Institution of Washington, Cold Spring Harbor, L. I.*)

The black pigment of the periodical cicada (*Tibicen septendecim* L.) is shown to be produced by the interaction of a chromogen and an oxidase of the tyrosinase group. Coloration proceeds after death but does not produce the normal uniform coloration, since, apparently, the tyrosinase is secreted together with the new cuticula, and after death this secretion ceases.

In the note on chemical anti-oxidases the suggestion is made that, perhaps, dominant whites are due to the presence of aromatic compounds carrying two hydroxyl groups in meta position to each other. It was noted that tyrosin did not produce the typical coloration in the presence of tyrosinase when orcin, resorcin, or phloroglucin—all meta-di-hydroxyl benzol derivatives—were present in the solution. This result was, apparently, caused by the tyrosinase being affected in the same manner as though an anti-oxidase were present, for proof was given that the tyrosin had not united chemic-

ally with the *m*-di-hydroxyl compound, and data were also given which makes it appear very improbable that the cause lies in a more rapid oxidation of the orcin, etc., to colorless derivatives. The only other alternative is that the action is of the same nature as that of a true anti-oxidase. If, therefore, through some body process, an additional hydroxyl were added to tyrosin adjacent to the alkyl chain, a compound would result which should not give colors with tyrosinase, nor allow colors to be produced even though tyrosin were present. Such a situation would produce dominant whites.

Effect of the Quantity of Protein Ingested on the Nutrition of Animals

II. ON THE WEIGHTS OF SOME VITAL ORGANS FROM LAMBS

W. E. CARROLL AND A. D. EMMETT

(*Laboratory of Physiological Chemistry, Department of Animal Husbandry, University of Illinois, Urbana, Ill.*)

Twenty-one Shropshire lambs of practically the same age and type and of very similar ancestry were divided into three lots of seven animals each. Lots I, II, and III were fed respectively on what was designated as low, medium, and high protein planes. A careful record was kept of the nutrients actually consumed. At the end of the feeding period, detailed slaughter tests were carried out. All of the various organs and parts from the twenty-three animals were weighed. Further, some of the special organs were preserved for chemical and histological studies.

The weights of the brains, kidneys, liver, and heart show, that with the exception of the kidneys, the feed has little or no effect upon the development of the organs as far as their weight is concerned. The kidneys seem to increase directly with the protein fed—the average weights for the three lots being 95.7, 110.1, and 122.3 grams respectively. Individuality seems to be a very great factor. In fact, if we apply mathematical methods as suggested by Rietz and Mitchell,¹ individuality is found to be a greater factor than feed, excepting in the case of the kidneys.

¹ Rietz and Mitchell: *Journal of Biological Chemistry*, 1910, viii, p. 297.

If the weights of these organs be calculated on the basis of one hundred pounds live weight, the averages for the three diets show that the brains and heart vary inversely as the protein fed, and that the kidneys vary directly. The data for the liver tend to show that Lot I is lower than either of Lots II or III, there being practically no difference between the weights for Lots II and III.

Further study is being made along this line to ascertain if possible the relative influence of feed and individuality.

Effect of the Quantity of Protein Ingested on the Nutrition of Animals

III. ON THE ASH AND TOTAL PHOSPHORUS FROM LAMBS

R. H. WILLIAMS AND A. D. EMMETT

(Laboratory of Physiological Chemistry, Department of Animal Husbandry, University of Illinois, Urbana, Ill.)

The special object was to study the influence of different amounts of protein in the feed upon the ash and phosphorus content of muscular tissues of lambs, fed under known conditions. In this particular case the leg of mutton cut was selected from twelve of the twenty-one animals referred to above by Carroll and Emmett.

The data show that (a) upon the fresh basis, the average percentage of ash tends to vary inversely as the amount of protein fed, and that the per cent. of total phosphorus is practically the same for the medium and low protein lots but that the value for the high protein lot is greater. (b) On the water- and fat-free basis, the percentage of total ash in the muscular tissue is almost the same for the high and medium protein-fed animals but higher for those fed on the low protein plane. The total phosphorus seems to vary directly with the amount of protein fed, the difference between the low and high lots being much smaller than that between the medium and high ones. (c) The per cent. of phosphorus in the ash shows that the ash contained more phosphorus as the amount of protein feed increased. This study is being continued with other muscular tissues from lambs, and with pigs.

Effect of the Quantity of Protein Ingested on the Nutrition of Animals

IV. ON THE CREATIN OF FLESH OF SWINE AND LAMBS

W. E. JOSEPH AND A. D. EMMETT

(Laboratory of Physiological Chemistry, Department of Animal Husbandry, University of Illinois, Urbana, Ill.)

The pigs in this experiment were the same as those described by Carroll and Emmett¹ in their study of the physical constants of fats. The lambs were the same as those described in paper II of this series (p. 110). The ham, shoulder, and side cuts were used in the case of the pigs and the leg of mutton cuts from the lambs.

From the chemical data, the per cent. of creatin in the water- and fat-free flesh of the ham and side cuts and also of the composite of the flesh of the ham, shoulder, and side cuts shows, in general, slight tendencies toward variations with the amount of protein fed. The percentage values for the shoulder cut, on the other hand, show the opposite tendency, the medium and high protein-fed lots being nearly the same and the low one distinctly greater. In the flesh of the leg of mutton, the average values for the three lots are practically the same.

Considering the per cent. of creatin nitrogen in the soluble nitrogen, a uniform variation is found in the cuts from the swine. The greatest value is found in the high protein lot and the least in the medium protein lot. In the leg of lamb-cuts, there is a uniformly decreasing average percentage with decreasing amounts of protein fed. The maximum differences in both cases are less than the difference between individuals within the lots.

On the whole the data seem to indicate slight differences between the three lots. However, the differences between individuals within the lots are such that no definite conclusions can be drawn at this time as to the effect of the quantity of protein in the feed on the creatin content of flesh. Differences between individuals seem to be

¹ Carroll and Emmett: Proceedings of the American Society of Biological Chemists, 1911, ii, p. 17; Journal of Biological Chemistry, 1911, ix, p. xxiii.

sufficiently well brought out to warrant the statement that more attention should be given to the number of animals, and to their selection.

A Cage Designed for Metabolism Experiments on Goats

A. R. ROSE

(New York Agricultural Experiment Station, Geneva, New York.)

In this station it was found most practical, when using the cow in metabolism experiments, to keep men constantly on the watch to collect the excreta. This method is laborious and expensive, and a smaller animal which could be caged easily was sought as a substitute for the unwieldy cow. For this purpose the goat serves admirably, and it is rather remarkable that an animal with so many qualifications for metabolism work has received so little attention. The goat is of convenient size to be readily handled, and it takes rations and yields excreta of very satisfactory bulk and might very well represent the herbivora in animal experimentation. It becomes quickly at home in the cage and adjusted to the demands of the investigator.

The cage consists essentially of an elevated wooden box, with gratings in the upper part, to admit light and air. Inside wooden walls are covered by galvanized sheet iron. One side is attached only at the top by means of hinges, and forms a door to admit or remove the goat, and for convenience in milking.

The floor is a heavy wire screen with wires sufficiently far apart to let all waste pass through, yet allowing five wires for each foot to stand upon.

Under the screen, at the front end, is a pan to collect any food dropped in eating. Under the rest of the floor is the device for separating the excreta from one another, consisting of two galvanized sheet-iron parts, the hopper and the urine pan. This hopper terminates in a trough leading toward the front end of the cage. This trough has at the point of junction with the hopper, an opening in its bottom protected by strands of wire, by which the dung pellets coming down the hopper are deflected into a suitable remov-

able receptacle standing on the floor under the front end of the cage. The urine passes through this hole into a shallow pan suspended from the hopper trough, immediately beneath. This pan has an elongated spout, leading forward, through which the urine flows into another receptacle standing on the floor beside the one provided for the dung.

The cage is simple in construction. It was made by local carpenters with the aid of a tinsmith, at a cost of thirty-seven dollars. The complete cage occupies a floor space of about two by four feet, is seven feet high and can be easily carried by two men. The cage is equally applicable to studies on sheep.

On the Lipins of the Heart Muscle of the Ox

JACOB ROSENBLOOM

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.)

MacLean and Williams¹ have found that the essential fat of the liver has the properties of phospholipin. They think it probable that the fatty matter from certain other organs is of the same nature. They find by extraction of the liver with ether and alcohol, at room temperature, that 84 per cent. of the total extract is phospholipin in quality, whereas, if the extraction is carried out at the temperature of the *boiling* solvent, only about 40 per cent. of the extract partakes of the properties of phospholipin. MacLean and Williams believe that such treatment with the *boiling* solvent causes a cleavage of the tissue phospholipin, with a consequent increase in the amount of neutral fat in the extract.

In a study of the lipins of the heart muscle of the ox, practically identical percentages of neutral fat and phospholipin were found by the writer in the ether and alcohol extracts which had been obtained by treatment with the respective solvents at room temperature and also at their *boiling* temperatures. It is possible, however, that the ether and alcohol extracts of the liver contain substances of a lipin nature which are more easily decomposed than those in similar extracts of heart muscle.

¹ MacLean and Williams: Biochemical Journal, 1909, iv, p. 455.

The Effect of Pregnancy on the Lipins of the Ovary and Corpus Luteum of the Cow

JACOB ROSENBLOOM

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.)

A comparative study of the amounts of neutral fat, fatty acid, lecithan and cholesterol, in ether and alcohol extracts of the ovary and corpus luteum of the cow, showed that pregnancy had no effect on the respective proportions in which these substances appeared in the extracts.

The Relation of Permeability to Fertilization of the Ovum

E. P. LYON AND L. F. SHACKELL

(United States Fish Commission, Beaufort, N. C.)

Comparative chemical analyses tend to show that ions such as Ca, Mg and SO_4 pass more readily out of fertilized than unfertilized echinoderm (*Arbacia*) eggs when the latter are suspended in an $m/2$ NaCl solution.

When thick suspensions of eggs are fertilized in sea-water, subsequent analyses both of the eggs and of the supernatant sea-water indicate that a considerable amount of Ca enters the eggs at the time of fertilization.

Although Lyon had previously shown that under otherwise equal conditions, fertilized eggs (*Arbacia* and *Toxopneustes*) were able to catalyze hydrogen peroxide more rapidly than unfertilized, more recent experiments, using *Arbacia* eggs and sperm disintegrated by shaking with finely powdered glass, have shown that disintegrated eggs plus disintegrated sperm, disintegrated eggs plus fresh sperm, or fresh eggs plus disintegrated sperm produce only additive catalytic effects.

The more rapid staining of fertilized than unfertilized *Toxopneustes* eggs with methylene blue and dahlia was shown to be due to the entrance of a greater quantity of stain in the first instance

by cytolyzing with distilled water equal quantities of fertilized and unfertilized stained eggs and making up the liquids to equal volumes. In each instance the liquid from the fertilized eggs showed a strikingly darker color.

These results indicate a sudden increase of permeability in echinoderm eggs upon fertilization.

The Stability of the Photogenic Material of the Lampyridæ and its Probable Chemical Nature

F. ALEX. MCDERMOTT

(Hygienic Laboratory, Public Health and Marine-Hospital Service, Washington, D. C.)

The photogenic compound present in the Lampyridæ is much more stable towards atmospheric oxygen than has usually been thought, especially when dried out of contact with air; it presents many points of similarity to other known biologic products; from embryologic and chemical considerations it appears probable that it is a lipoid or a lecithin.

The Gases of Swiss Cheese

WM. MANSFIELD CLARK

(Dairy Division Research Laboratories, Bureau of Animal Industry, Washington, D. C.)

An apparatus is described for isolating the gas from the "eyes" of Swiss cheese. Successful isolation has been accomplished and the gas analyzed. The constituent gases vary with the age of the cheese. Further experiments are being conducted with the view of determining, if possible, whether the nature of the gases and their ratios can be used to determine the species of bacteria which are thought to be responsible for the formation of the eyes.

The Brine-Soluble Compound Found in Cheese

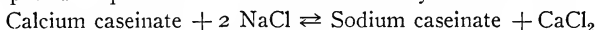
L. L. VAN SLYKE AND ALFRED W. BOSWORTH

(*Chemical Laboratory, New York Agricultural Experiment Station, Geneva, N. Y.*)

Investigations which have been conducted in this laboratory during recent years have shown that in the ripening of cheddar cheese a form of protein is always produced which is soluble in a 5 per cent. sodium chloride solution. The presence of this brine-soluble compound was shown to be connected in some way with the development of acid in the cheese. The compound was at first erroneously supposed to be paracasein-monolactate and later free paracasein. In recent work it was noticed that calcium was always to be found associated with this brine-soluble compound when it was separated from the other cheese constituents by extraction with a solution of sodium chloride (c. p.—free from calcium), after first removing the water-soluble constituents.

This brine-soluble compound is always present in cheddar cheese. In a cheese two years old 40 per cent. of the nitrogen was present in this form. It is also a fact that in cheddar cheese the calcium is never completely extracted by water, part of it always being found in the brine extract. In camembert cheese, however, the reverse is found. After the first few hours this cheese contains no brine-soluble compound and all the calcium is found in the water extract. The brine-soluble compound is formed in this cheese, but, owing to the method of making, more acid is allowed to develop than in cheddar cheese and, as a consequence, the brine-soluble compound loses its calcium and thereby becomes free paracasein, which is insoluble in brine solution.

We believe that, according to the evidence in hand, the following equation represents the reaction which takes place where the compound in question is taken into solution by a salt solution:



We believe that the mass action, thus represented, is also connected with the precipitation produced upon adding calcium chloride to the brine-soluble compound after its solution has been freed from excess of chlorides by dialysis.

The Influence of Sodium Chlorid on the Precipitability of Casein by Acetic Acid and its Bearing on the Partition of Nitrogen in Butter

WILLIAM N. BERG

(Dairy Division Research Laboratories, Bureau of Animal Industry, Washington, D. C.)

Acetic acid as a precipitant for casein in milk, buttermilk, etc., has been the subject of many careful researches. While the different methods of precipitation may differ in certain details, they are alike in principle. The milk, or other casein-containing fluid, is diluted to a convenient volume and acetic acid is added in amount sufficient to flocculate the casein completely. Thus, if 200 c.c. of buttermilk (or milk) are diluted to nearly 500 c.c., the addition of 5 c.c. of 10 per cent. acetic acid will flocculate the casein. Such a suspension of flocculent casein filters rapidly and gives a clear filtrate. If to a second 200 c.c. portion of the same sample of buttermilk 36 grams of sodium chlorid are added before diluting with water, the addition of 5 c.c. of 10 per cent. acetic acid does not flocculate the casein. The sodium chlorid very profoundly influences the precipitability of the casein because none is precipitated under these conditions. Not until three or four times the usual amount of 10 per cent. acetic acid is added (15-20 c.c.) does the casein flocculate, permitting rapid filtration.

The above described effect of the sodium chlorid was noticed in the course of a study of the possible proteolytic changes taking place in cold storage butter. Butter usually contains casein (1 per cent.), sodium chlorid (3 per cent.) and water (16 per cent.) in such proportions that when butter is melted, allowed to stand, and the clear supernatant fat poured off, a thick, viscid fluid (curd solution) remains, which is mixed with several per cent. of fat and contains approximately 6 per cent. of casein and other nitrogenous substances, and 20 per cent. of sodium chlorid.

If the curd solution obtained from butter be treated with water and acetic acid as if it were so much milk, flocculation does not

take place, nor can the milky mixture be filtered for quantitative purposes by any of the ordinary methods.

Previous investigators used other precipitants such as ferric chlorid, tannic acid, copper sulfate, etc. For our purpose these were objectionable because they precipitate not alone the casein and other proteins present, but their immediate digestion products as well. This leaves too little nitrogen in the filtrate for reliable analytic results.

These difficulties are obviated by the use of the larger amounts of acetic acid, as above indicated.

The Estimation of Creatin

STANLEY R. BENEDICT

(Cornell University Medical College, New York City.)

Twenty c.c. of urine (or a volume equal to twice the amount which will be required for an accurate creatinine reading) is treated with 20 c.c. of approximately normal hydrochloric acid and the mixture boiled nearly to dryness in a beaker or open flask. After the mixture has almost reached dryness it is placed in a boiling-water bath, and allowed to remain there for about five minutes after the residue is approximately dry. With the aid of warm water the residue is then washed into a 50 c.c. volumetric flask, the mixture cooled, and 5c.c. of 8 to 10 per cent. basic lead acetate solution added, and the mixture diluted to exactly 50 c.c., and mixed by shaking. The mixture is filtered through a dry filter into a dry beaker and 25 c.c. of the filtrate used for the colorimetric determination as in Folin's process, save that 6 c.c. of 10 per cent. alkali are employed, which should best contain also 5 per cent. of Rochelle salt. This process has the great advantage that in the conversion of the creatin less pigment is produced than in former methods.

The Determination of Calcium in the Presence of Phosphates and Magnesium

F. H. MCCRUDDEN

(Hospital of the Rockefeller Institute for Medical Research,
New York City.)

Summary of the results of Part I. I shall summarize the results by describing details of the methods which may best be used.

Special solutions needed: 2½ per cent. oxalic acid solution; 3 per cent. ammonium oxalate solution; 20 per cent. ordinary acetate solution.

1. The solution containing calcium (it may also contain magnesium and phosphate and small amounts of iron) is brought to a volume of from 75 to 150 cubic centimeters. Concentrated ammonia solution is added drop by drop until the solution is just alkaline. This point may be recognized either by the precipitation of calcium and magnesium phosphate, if present in any amount, or by change in color of an indicator which may be added.

2. Concentrated hydrochloric acid is added drop by drop until the solution is just acid. This may be recognized by the disappearance of the precipitate of phosphate if present, or by the change in color of an indicator. If iron is present, an indicator (alizarine red) should be used, for in this case the precipitate of phosphate of iron does not disappear as soon as the solution becomes acid. The change in color of the indicator and not the disappearance of the precipitate is the end point in this case.

3. Ten drops of concentrated hydrochloric acid (specific gravity 1.20) are added. It is best to ascertain, first, that ten drops of the concentrated acid with the dropper used are equivalent to 10 c.c. of $n/2$ hydrochloric acid within about 10 to 15 per cent.

4. Ten cubic centimeters of 2½ per cent. oxalic acid are added.

5. Either one of two methods of procedure may be used—*a* or *b*. (*a*) The solution is boiled until the precipitated calcium oxalate is coarsely crystalline,¹ and then an excess of 3 per cent. ammonium oxalate should be slowly added to the boiling solution and

¹ If but little calcium is present, none precipitates at this point.

the boiling continued until the precipitate is coarsely crystalline.² (b) The flask is closed with a rubber stopper and shaken vigorously for ten minutes. After this an excess of 3 per cent. ammonium oxalate is added.

6. The solution is cooled to the room temperature and 8 c.c. of 20 per cent. sodium acetate added. (In the case of feces-ash, add 15 c.c. of acetate solution.)

7. The solution may be either (a) allowed to stand over night, or (b) stoppered and shaken vigorously for ten minutes.

8. The calcium oxalate is filtered off on a small ash-free filter and washed free from chloride with 0.5 per cent. ammonium oxalate solution.

9. One of two things may be done here: (a) The precipitate and filter are dried, incinerated in a weighed platinum crucible to CaO, and brought to constant weight before a blast lamp. (b) The excess of ammonium oxalate from the washing fluid is removed from the calcium oxalate precipitate by washing four times with cold distilled water, each time filling the filter about two-thirds full and allowing the wash water to drain off completely before adding more. A hole is punched in the point of the filter paper with a glass rod and the calcium oxalate washed down into a flask with a stream of water from a wash bottle. The fluid is brought up to about 50 c.c. in bulk and 10 c.c. of concentrated sulphuric acid are added. The oxalate is titrated in the hot solution (40°–60° C.) with standard potassium permanganate solution. If magnesium is to be determined in the filtrate, the determination may be carried out as described in my earlier paper on the subject.

Summary of the results of Part II. The results may be summarized by describing the details of the method found to be best.

Solutions needed: 2½ per cent. oxalic acid solution; 20 per cent. sodium acetate solution.

1. If the urine is alkaline it is made neutral or slightly acid so that all calcium is in solution.

2. The neutral or slightly acid urine is filtered to free it from all detritus and sticky material that may later interfere with filtration of calcium oxalate.

² If but little calcium is present it is not necessary to add oxalate.

3. Two hundred c.c. of urine are used for analysis. If the urine is just barely acid to litmus paper, as normal urine usually is, 10 drops of concentrated hydrochloric acid are added.

If the urine is strongly acid, it may first be made just alkaline by the addition of ammonia drop by drop and then just acid by the addition of concentrated hydrochloric acid drop by drop. As soon as the urine is alkaline, a precipitation of phosphates occurs. As soon as it is made acid, the precipitate of phosphates disappears. One or two drops of acid or ammonia should make the change distinct. If this is not so, either because the urine is turbid or contains very little calcium, litmus paper should be used to determine the change. After the urine has been made just barely acid in this way, 10 drops of concentrated hydrochloric acid (specific gravity 1.20) are added.

4. Ten c.c. of $2\frac{1}{2}$ per cent. oxalic acid solution are next added.

5. Eight c.c. of 20 per cent. sodium acetate solution are added.

6. The urine is allowed to stand over night at the room temperature.

7. The calcium oxalate is filtered and washed free from chlorides with 0.5 per cent. ammonium oxalate solution, until free from chlorides.

8. (a) The precipitate may then be dried, incinerated, heated in the blast lamp to constant weight and weighed as CaO . (b) If the precipitate is free from uric acid, the calcium oxalate may be determined by titration with standard permanganate solution instead of by weighing. In this case the ammonium oxalate from the washing fluid is first removed from the precipitate by washing four times with cold distilled water, filling the filter about two thirds each time and allowing the wash water to drain completely. A hole is then punched in the point of the filter. The calcium oxalate is washed through into a flask, 10 c.c. of concentrated sulphuric acid are added and the oxalate titrated with standard permanganate solution at a temperature of from 40° to 60°C .

Methods of Estimating Moisture in Tissues

WALDEMAR KOCH

(*Laboratory of Pharmacology, Chicago University.*)

With valuable biological material it is sometimes desirable to make water estimations and the estimations of the other constituents on the same sample. As there is danger of decomposing the constituents by the high temperature employed for drying in the official method, a comparison of this method with the one devised some years ago¹ and used in this laboratory was made. The results are recorded in the following table.

	W. 8. Direct with Alcohol.	W. 2r. Dried by Heat at 95° C.
Proteins	48.5	47.5
Phosphatids	21.6	16.3
Cerebrosids	8.8	9.4 ²
Sulphatids	3.6	4.3 ²
Undetermined lipoids	8.2	11.0 ³
Organic and inorganic extractives	9.3	11.6
	100.0	100.1
Lipoid P in per cent. of total P...	62.5	53.6

The Preparation of Tissue for Toxicological Examination

JAMES P. ATKINSON

(*Chemical Laboratory of the Department of Health,
New York City.*)

The finely minced tissue is digested with artificial gastric juice. The solution is filtered and extracted for alkaloids in the usual way. After this extraction the material is evaporated with nitric acid and then examined for metallic poisons. This method has three advantages: (1) The examination may be completed within three days, (2) less personal attention is required, and (3) the tissue is completely broken down and therefore allows a better

¹ Koch: Journal of the American Chemical Society, 1909, xxxi, p. 1335.

² Variation due to difference of age.

³ Increase due to fatty acids from destruction of phosphatids.

solution of the alkaloids than by extracting the minced tissue with acid alcohol.

Studies of Water Absorption by Colloids¹

WILLIAM J. GIES

(Laboratory of Biological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.)

Fischer's theory regarding the influence of acids in the production of edema is based upon observations of facts which have been demonstrated repeatedly, but the experimental results do not, in the writer's opinion, justify Fischer's sweeping application of them to the explanation of all pathological edemas.² Experiments with enucleated eyes (from dogs, rabbits, and chickens, in *solutions* of combined acids) similar to those already described,³ failed to yield edematous results, but emphasized the need for experiments on *solutions* of biological colloids, such as serum and lymph. Fischer's theory is based upon the results of experiments on *solid* masses in large excesses of acid solutions. He has not shown that his experimental conditions are closely analogous to the natural ones in edema.

Experiments along these and collateral lines are now in progress.⁴

¹ These studies are members of a projected series on *proteins and their combining qualities*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm and the nature of the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in this laboratory, and under the auspices of the George Crocker Special Research Fund.

² Fischer: *Edema: A study of the physiology and the pathology of water absorption by the living organism*. Pp. 209. 1910.

³ Goodridge and Gies: *Proceedings of the Society for Experimental Biology and Medicine*, 1911, viii, p. 106.

⁴ This abstract is made very brief and general because the writer's discussion of Fischer's theory, at the June meeting of the Columbia University Biochemical Association, will be published in the December issue of the *BIOCHEMICAL BULLETIN* as a part of a symposium on edema, and includes all that might be stated here.

On the Diffusibility of Biological Substances Through Rubber¹

WILLIAM J. GIES

(Laboratory of Biological Chemistry, of Columbia University, at
the College of Physicians and Surgeons, New York.)

The writer and his associates have found that many ether-soluble substances of biological origin, such as fat and cholesterol, pass readily from ether solutions through rubber membranes into ether when the mechanical conditions for such diffusions are favorable. Lecithans appear to be wholly indiffusible.

Many substances which are soluble in fatty oils, chloroform, alcohol, acetone, ethyl acetate and other solvents of similar powers, or in mixtures of such solvents, promptly diffuse through rubber under suitable conditions. Collodion is one of the products which appears to be indiffusible under such circumstances. When an ordinary ethereal solution of collodion (containing 24 per cent. of alcohol) is dialyzed in a rubber condom against ether in a closed vessel, the alcohol rapidly passes to the exterior and the collodion gradually gelatinizes. Liquid accumulates in the bag under these conditions.

Various inorganic substances diffuse through rubber under the conditions mentioned above. Ferric sulfocyanate readily passes from ether solution through rubber into ether.

The writer inaugurated these studies, with Dr. Rosenbloom's cooperation, in the hope of devising improvements in the methods for the isolation of lipins (see page 51). Our findings for biological substances accord with the results of Kahlenberg's experiments with rubber membranes in other connections.² The work

¹ These studies are members of a projected series on *physico-chemical conditions in the cell*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm and the nature of the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in this laboratory, and under the auspices of the George Crocker Special Research Fund.

² Kahlenberg: Transactions of the Wisconsin Academy of Sciences, Arts and Letters, 1904, xv, p. 209.

is progressing along several lines, especially with reference to methods of isolation and purification, and to osmosis.¹

Aging of Flour and its Effect on Digestion

J. A. WESENER AND GEORGE L. TELLER

(Columbus Laboratories, No. 13 N. State St., Chicago, Ill.)

The aging of flour by the use of oxides of nitrogen, and similar agents artificially supplied, is simply a hastening of the process which is continually going on in the storage of flour. Flours exposed to the air for greater or less time, according to conditions, take up nitrogen oxides from the air and lose their color just the same as when treated with the bleaching gases. Alkali corn starch exposed to the air, or even when packed in a paper carton and sealed with paper wrapper, takes nitrites rapidly from the air. Vaughan's experiments indicate that the product formed by the union of the coloring matter of flour with the bleaching gases is not poisonous and that it gives the Liebermann nitroso test.

The nitrite reaction in flour seems to be due entirely to direct union between the coloring matter and nitrogen oxid.

Nitrites do not interfere with diastase in its action on starch, even when present as sodium nitrite to the extent of one part in one thousand.

Nitrous and nitric acid do not inhibit the action of peptic digestion, and may wholly replace hydrochloric acid in this essential first stage of digestion.

While pancreatic digestion will not take place in the presence of free acids, it is not inhibited by the presence of relatively large quantities of nitrites, nor is its action restrained on protein which has been previously subjected to the action of appreciable quantities of nitrous and nitric acids.

No experiments have demonstrated the presence in commercially bleached flour of either mineral nitrite, nitrous acid or nitric acid.

¹Rosenbloom and Gies: Proceedings of the American Society of Biological Chemists, 1911, ii, p. 8; Journal of Biological Chemistry, 1911, ix, p. xiv; Rosenbloom and Gies: Proceedings of the Society for Experimental Biology and Medicine, 1911, viii, p. 71; Gies: *Ibid.*, p. 73; Goodridge and Gies: *Ibid.*, p. 74; Sidbury and Gies: *Ibid.*, p. 104; Boas and Rosenbloom: *Ibid.*, p. 132.

The Occurrence of Lipase in the Fat of the Common Fowl—*Gallus Domesticus*

M. E. PENNINGTON AND J. S. HEPBURN

(Food Research Laboratory of the Bureau of Chemistry, U. S. Department of Agriculture, Philadelphia.)

If a chicken be kept hard frozen or at the temperature of the room, or at any temperature between these two extremes, the acidity of the fat increases, as has been shown in previous publications from this laboratory. Since the fat-splitting enzyme, lipase, is found in many plant and animal tissues, this investigation was undertaken to determine if lipase is present in the crude fat of chickens. The technique is fairly simple. The crude abdominal fat is passed several times through a meat chopper, and its acidity is determined. A weighed sample of the ground fat is triturated in a mortar with sand, and then extracted with ten times its weight of water. Fifty c.c. of the aqueous extract and 1 c.c. of an ester (ethyl acetate, butyrate or benzoate, or amyl salicylate) are mixed, the solution is made neutral to phenolphthalein and incubated at 40° C. for periods of time varying between 24 and 168 hours—usually 72 hours. Toluol is used as a bactericide. Fifty c.c. samples of the aqueous extract are boiled, then run as blank experiments in exactly the same manner as were the determinations proper. At the end of the incubation both determinations and blank experiments are titrated; the increase in acidity of the determination proper over the blank is due to the action of lipase.

This research has demonstrated the presence of lipase in the crude abdominal fat of fresh chickens retaining the animal heat, and of chickens kept at temperatures from that of the room to that of the "freezer" for varying periods of time. The highest acidity of the crude fat, and the greatest activity of the lipase, occurred in chickens which had been kept hard frozen for sixteen months, or which had been permitted to putrefy at room temperature. The lowest acidity of the crude fat, and the least activity of the lipase, were found in a fresh chicken still retaining the animal heat. Apparently in fresh birds the enzyme is present as a zymogen, which is converted into the active form as the chicken ages after death.

Deterioration in Eggs as Shown by Changes in the Moisture Content

A. D. GREENLEE

(Food Research Laboratory of the Bureau of Chemistry, U. S. Department of Agriculture, Philadelphia.)

Eggs contain a high percentage of moisture when fresh—white about 88 per cent., and yolk about 48 per cent. This percentage of moisture is constantly changing, due both to a loss to the external atmosphere by evaporation and also to internal rearrangement. The yolk absorbs water from the white. This change increases with the temperature and time, and when carefully measured it becomes a good index of the condition and probable age of the egg. By test experiments on a uniform lot of eggs, held at a constant temperature and analyzed at short intervals of time, the rate of change of moisture content can be determined and plotted and by means of the subsequent formula derived, the condition of any lot of eggs can be predicted from the first analysis for any given date within the holding period.

By a further extension of the work now in progress it is hoped that the age and past history of the egg can be deciphered from a determination of the percentage and relative distribution of the moisture.

The Oxidation of Chicken Fat with Hydrogen Peroxide

J. S. HEPBURN

(Food Research Laboratory of the Bureau of Chemistry, U. S. Department of Agriculture, Philadelphia.)

When light, air, heat and enzymes act on fats and oils, the various constants undergo changes, and an increase in saponification number is usually accompanied by a decrease in Hehner number, and *vice versa*. This phenomenon is due chiefly to the oxidation of the unsaturated acids at the double bonds. However, when chickens are kept hard frozen, both the saponification number and

the Hehner number experience simultaneous change in the same direction. Thus nine analyses give a mean saponification number 172.9 and a mean Hehner number 81.27 for fresh roasters, while three analyses of undrawn roasters, kept hard frozen for 16 months, give a mean saponification number 194.9 and a mean Hehner number 91.67; the two constants have increased at the same time. This species of fat decomposition must be due to oxidation of the carbon chain at or near the terminal carbon atoms. The recent work of Dakin upon the oxidation of saturated fatty acids by means of hydrogen peroxide, led to the present research.

Fat was extracted from chickens and analyzed. The extracted fat was heated on the water-bath for seven hours with three per cent. solution of hydrogen peroxide—six molecules of peroxide were used for each molecule of fat; the fat was then separated from the aqueous layer, washed free from peroxide with boiling water, filtered through paper and analyzed. The acidity always became higher; the iodine number usually decreased, though it occasionally increased. The saponification number and the Hehner number almost invariably increased simultaneously, hence dilute hydrogen peroxide at the temperature of the water-bath produces in chicken fat the same change as occurs in that fat *in situ* while hard frozen.

When oleic acid and stearic acid were oxidized in this manner, their saponification numbers decreased. This change is similar to that undergone by the fat of chickens kept hard frozen for periods of four months, at the end of which time both the saponification number and the Hehner number are lower than in the fat of fresh birds.

Detection and Role Played by Poly-Atomic Phenols Occurring in Apples as Glucosides

H. P. BASSETT

(*Delaware Agricultural Experiment Station, Newark, Del.*)

In apples there is a glucoside resembling phloridzin. There is present also an enzyme which hydrolyzes it, liberating a polyatomic phenol. From the phenol by the action of an oxidizing enzyme

a phlobaphene is formed. This oxidase reaction renders the fluid germicidal. It is suggested that this has a protective value for the fruit.

Observations on the Deterioration of Maize

O. F. BLACK AND C. L. ALSBERG

(*Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.*)

The discovery by the authors of the formation of penicillic acid by *Penicillium puberulum* Bainier, has enabled them to improve the method for detecting spoiled corn by means of the "ferric chloride" or "phenol reaction of Gosio." The dry meal is extracted with chloroform and the extract is tested directly with ferric chloride. The delicacy of the reaction is thereby greatly increased.

An Incubator for Moderate Temperatures

A. M. BUSWELL AND RALPH H. MCKEE

(*Department of Chemistry, University of Maine, Orono, Me.*)

The incubator uses, without the aid of a relay, a 110 volt A. C. current for the heating and the regulation of the current. The expanding liquid of the regulator is alcohol, the capillary U tube outlet being filled with mercury. Five wires are sealed into the capillary tube and the resistances attached so that the voltage drop, as the mercury passes a sealed-in wire, will be but twenty volts. This is below the arcing voltage and consequently no carbonization occurs and practically no gas is formed by the make and break. The lights used for heating are in series with the mercury and such resistances as are pushed in by the expanding alcohol. Without attention the incubator kept between 36.5° and 37.5° for two months.

The Absorption of Inorganic Salts by Living Protoplasm

W. J. V. OSTERHOUT

(*Department of Botany, Harvard University, Cambridge, Mass.*)

The usual method of determining osmotic pressure by plasmolyzing in salts of Na and K is very erroneous. Salts of Ca give more nearly the true osmotic pressure.

Since one substance may greatly affect the penetration of another, it is unsafe to use the common method of adding a toxic to a non-toxic substance and judging the penetration of the former by the plasmolytic action of the mixture.

It is possible to state which salts penetrate and at what rate of speed, and also how various salts affect the permeability of the plasma membrane.

From the data obtained we have a definite clue to the nature of the plasma membrane. Since all the salts studied penetrate, it seems certain that the membrane cannot be lipid because these salts are not soluble in lipid. Its behavior toward balanced solutions (together with other facts) indicates unmistakably that the membrane is protein in nature.

Antagonistic salts such as NaCl and CaCl₂ hinder or prevent each other from entering. To such an extent is this true that by choosing solutions of NaCl and of CaCl₂ which are not quite strong enough to plasmolyze, we produce by mixing them together a solution which plasmolyzes strongly.

The fact that these salts hinder or prevent each other from entering may explain why they act as antidotes to each other. But since they may eventually penetrate to some extent we must attach importance to their effect on the protoplasm within the cell as well as to their effect on the plasma membrane. These two effects may be very similar. (*Science*: 1911, xxxiv, p. 189.)

Carbohydrate Esters of the Higher Fatty Acids

WALTER R. BLOOR

(*Washington University Medical School, St. Louis, Mo.*)

Esters of mannitol with stearic acid were prepared and their properties described. One of them was fed to animals. It was found that about 50 per cent. was absorbed.

The Leaf-Oil of the Washington Cedar (*Thuja plicata*)

ROBERT EVSTAFIEFF ROSE AND CARL LIVINGSTON

(*Department of Chemistry, University of Washington, Seattle*)

The leaf oil of the Washington cedar (*Thuja plicata*) has not been examined chemically save by I. W. Brandel who published a short note on its composition (*Pharm. Review*, 26, 248). The investigation referred to being only in the nature of an approximate analysis, the authors thought it advisable to undertake a more detailed study of the oil, the results of which follow. It may be mentioned that the resultant conclusions differ very appreciably from those of Brandel (*loc. cit.*).

Steam distillation at 100°, of the leaves and twigs of *Thuja plicata* produced about 1 per cent. of a clear, light yellow oil which possesses the characteristic odor of cedar boughs. The following constants were found: D_{20} 0.913; $n_{D_{20}}$ 1.4552; $[\alpha]_{D_{20}}$ — 4.77; acid number, 0.518; ester number, 2.28; saponification number, 2.8; acetylation number, 8.8. An elementary analysis showed the absence of sulphur and nitrogen, while the percentage composition (C=78.6; H=10.4 per cent.) agreed very closely with that of a bicyclic ketone $C_{10}H_{16}O$ (calc. C=78.04; H=10.52 per cent.). The oil contained no phenols and was soluble in all proportions in anhydrous organic solvents and in 70 per cent. ethyl alcohol.

A fractional distillation under reduced pressure showed that 85 per cent. of the oil boiled between 100° and 110° under 40 mm. The small fraction (about 4 per cent.), boiling below 100°, 40 mm., gave D_{20} 0.851; $n_{D_{20}}$ 1.4609; $[\alpha]_{D_{20}}$ + 36.8, and was chiefly com-

posed of pinene, which was identified by the preparation of the nitroso-chloride, m.p. 103° . The main fraction was repeatedly distilled and yielded thus an oil boiling at $103-104^{\circ}$ under 40 mm., which was found to be thujone by the following constants and derivatives:

$$D_{20} \ 0.9152; \ ^nD_{20} \ 1.4530; \ [\alpha] \ D_{20} \ -11.58.$$

Derivatives: tribromide, m.p. $121-122^{\circ}$; semicarbazone, m.p. $186-188^{\circ}$ (from methyl alcohol); tanacetone keto-carbonic acid, m.p. $75-76^{\circ}$.

The reaction with hydroxylamine gave an oily oxime which crystallized only partially after standing for several months; which fact, together with the observed levo-rotation—a rotation, it may be noted, slightly higher than any previously recorded—further characterized the substance as α -thujone.

The fraction boiling from 100 to 103° , 40 mm. ($D_{20} \ 0.8975$; $^nD_{20} \ 1.4549$; $[\alpha]D_{20} \ -0.62$), was tested for fenchone, since that ketone has been isolated from the oil of *Thuja occidentalis*. Using Wallach's method—oxidation with potassium permanganate, steam distillation, further oxidation with concentrated nitric acid, and recovery of the unaltered fenchone by distillation in steam—only a few drops of oil were obtained which were heavier than water and in which no fenchone was found. An attempt to prepare the oxime of the ketone after removal of the thujone by oxidation was equally unsuccessful. The authors conclude, therefore, that fenchone is not present as stated by Brandel (loc. cit.).

The residue (about 5 per cent.) boiling above 110° , 40 mm. ($D_{20} \ 0.980$), was dark brown in color and had an odor of stewed prunes. This was hydrolysed with alcoholic potash, steam distilled, and fractionated. A light yellow oil was obtained whose constants show it to be tanacetyl alcohol ($D_{25} \ 0.9266$; $^nD_{25} \ 1.46207$; b.p.₇₅₇ $210-220^{\circ}$, $[\alpha]D_{25} \ +29.8$), probably present as acetate in the original oil.

From the above results, the authors conclude that the volatile oil of *Thuja plicata* is composed of 80-85 per cent. thujone, 3-5 per cent. pinene, 1-2 per cent. tanacetyl acetate, and 1-3 per cent. tantacetyl alcohol, leaving about 10 per cent. to be accounted for by loss due to formation of resin during distillation and to experimental waste.

Communications which have not been abstracted

DEMETHYLIZATION UNDER NORMAL AND PATHOLOGICAL CONDITIONS. I. CHRONIC ALCOHOLISM. William Salant and I. K. Phelps. (*Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.*)

ELIMINATION OF CAFFEIN IN THE URINE. William Salant and J. B. Rieger. (*Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.*)

EFFECT OF DIET ON RESISTANCE TO DRUGS. William Salant. (*Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.*)

IN MEMORIAM

CHRISTIAN ARCHIBALD HERTER

Minutes adopted unanimously by a rising vote at the meeting
of the Faculty of the College of Physicians and Surgeons,
of Columbia University, on January 16, 1911

"The Faculty of the College of Physicians and Surgeons desires to express formally its deep sorrow at the death of Christian Archibald Herter, late Professor of Pharmacology in this College.

"To some members of this body a close personal friend and professional associate for many years, Dr. Herter represented to the faculty at large a most valued colleague, whose sterling personal character and high scientific attainments elicited uniformly the sincere admiration and respect of all privileged to come into closer contact with him.

"Outside of the circle of the faculty, in the institution at large, and in the wider scope of his manifold activities, in and beyond New York, Dr. Herter stood preeminently as a representative of the true scientific spirit on which the modern advances of medical research, education, and practice depend.

"It seems peculiarly sad that his death occurred so near to the time when the College, which he served with such ability and keen interest in his work, is approaching a period in the development of its medical education and investigations, in which many of his proposals and ideals are to be realized and carried into effect. He would have been, if spared to his share in the work of the impending reorganization of the school, one of our most valuable forces in council and in practical execution.

"In expressing our grief and our profound sense of personal and institutional loss, we may also gratefully realize that Dr. Herter's influence and example are still actively with us, and of real aid in our endeavor to accomplish the work before us sanely, conscientiously, in the spirit of advancing science, and for the true betterment of our calling in all of its manifold relations to humanity."

SAMUEL W. LAMBERT, *Dean.*

WILLIAM HENRY WELKER, A.C., PH.D.,
Assistant Professor of Biological Chemistry in Columbia
University, 1911-

MEMORANDUM WHICH WAS PRESENTED TO THE FACULTY OF MEDICINE WITH DR. WELKER'S NOMINATION TO THE ASSISTANT PROFESSORSHIP

William Henry Welker was born in Red Hill, Pa., August 20, 1879. He received his early education in the public schools and prepared for college at Perkiomen Seminary and Ursinus College. He taught public school at Klinesville, Pa., during the terms of 1898-99 and 1899-1900, and graduated from Perkiomen Seminary in the spring of 1900. He entered Lehigh University in the fall of 1900 and graduated from Lehigh in 1904, receiving the degree of Analytical Chemist. He was undergraduate instructor in general chemistry at Lehigh in 1902-03 and 1903-04, and undergraduate instructor in physics in 1903-04.

He was appointed Assistant in Chemistry at Lehigh University at the time of his graduation there in 1904, but resigned during the summer of 1904 to accept the position of Assistant in Physiological Chemistry in Columbia University, at the College of Physicians and Surgeons. On Dec. 1, 1906, while occupying this position, he became, in addition, Pathological Chemist in Obstetrics at the Sloane Hospital for Women.

In the fall of 1907 Dr. Welker resigned his Assistantships at Columbia to accept the position of Demonstrator of Physiological Chemistry in the Medical School of the University of Pennsylvania, where he remained until the end of the academic year 1909-'10, when he resigned to accept appointments to the positions of Associate in Biological Chemistry at Columbia University and Pathological Chemist at the German Hospital. In January, 1911, he resigned the latter position in order to devote himself wholly to the work of the former in research under the auspices of the George Crocker



H. H. Helker

Special Research Fund. (His promotion to an Assistant Professorship in Biological Chemistry occurred last spring.)

Dr. Welker was chemist to the Board of Health of Allentown, Pa., during the summer of 1905. He was "Chemist-in-charge of the Port of Philadelphia" during the summer of 1910. He received the degree of Ph.D. from Columbia University in 1908.

Dr. Welker was one of the charter members of the American Society of Biological Chemists, which was established in 1906.

The appended summary presents a list of Dr. Welker's publications:

(The asterisks below indicate the publications from the Laboratory of Biological Chemistry, of Columbia University, at the College of Physicians and Surgeons.)

1905. *The influence of radium bromid on metabolism in dogs (with William N. Berg). *Proceedings of the Society for Experimental Biology and Medicine*, 2: 89; *American Medicine*, 9: 1030; *Science*, 21: 988; *Medical News*, 87: 526.—Report on the city water supply of Allentown, Pa. *Annual Report of the Board of Health of the City of Allentown, Pa.*, p. 14.

1906. *Experiments to determine the influence of radium bromid on protein metabolism in dogs (with William N. Berg). *Proceedings of the Section of Biological Chemistry of the American Chemical Society in affiliation with Section C of the American Association for the Advancement of Science*, *Science*, 23: 333 and *Proceedings of the American Association for the Advancement of Science*, 55: 327.—*On the composition and toxic properties of *Ibervillea Sonorae* (with Julia T. Emerson). *Proceedings of the American Association for the Advancement of Science*, 55: 331 and *Science*, 23: 336.—*A simple electrical annunciator for use in metabolism experiments and in connection with filtration, distillation and similar operations (with William J. Gies). *Proceedings of the Society for Experimental Biology and Medicine*, 3: 77; *Science*, 23: 980; *American Medicine*, n. s., 1: 160.—*Experiments to determine the influence of the bromids of barium and radium on protein metabolism in dogs (with William N. Berg). *Journal of Biological Chemistry*, 1: 371.

1907. *On the cause of a red coloration in the iodoform test for acetone when applied to distillates from urine preserved with thymol. *Proceedings of the American Society of Biological Chemists*, 1: 36; *Journal of Biological Chemistry*, 3: p. xxvii.—*A further study of

the chemistry and pharmacology of *Ibervillea Sonorae* (with Julia T. Emerson). *Proceedings of the American Association for the Advancement of Science*, 56-57: 442; *Science*, 25: 460.—* The effects of salts of some rare elements on seedlings (with Alice A. Knox). *Proceedings of the American Association for the Advancement of Science*, 56-57: 442; *Science*, 25: 461.—* Contribution to our knowledge of the effects of urinary preservatives on urinary analysis. *New York Medical Journal*, 86: 552.—* A simple electrical annunciator for use in metabolism experiments, and in connection with filtration, distillation and similar operations. *American Journal of Physiology*, 20: 358.

1908. * A study of the influence of potassium cyanide on the excretion of nitrogenous substances in the urine of dogs. *Proceedings of the American Association for the Advancement of Science*, 58: 276 and *Proceedings of the American Society of Biological Chemists*, 1: 125; *Journal of Biological Chemistry*, 4: p. xxxi.—* Notes on the chemical nature of egg cases of two species of sharks (with Louis Hussakof). *Proceedings of the American Society of Biological Chemists*, 1: 138; *Journal of Biological Chemistry*, 4: p. xlv.—* On some biochemical and anatomical changes induced in dogs by potassium cyanide (with Norman E. Ditman). *New York Medical Journal*, 88: 59.—* Some notes on the chemical composition and toxicity of *Ibervillea Sonorae* (with Julia T. Emerson). *Journal of Biological Chemistry*, 5: 339.

1909. * Studies of the influence of various dietary conditions on physiological resistance. II. The influence of different proportions of protein in the food on the partition of urinary nitrogen after dosage with potassium cyanide (with Norman E. Ditman). *Dissertation*. Pp. 30. Columbia University.—A disturbing factor in Barfoed's test. *Proceedings of the American Society of Biological Chemists*, 1: 180; *Journal of Biological Chemistry*, 6: p. xxxiii.—* Deficient oxidation in its relation to the etiology, pathology and treatment of nephritis (with Norman E. Ditman). *New York Medical Journal*, 89: 1000-1006; 1046-1052; 1091-1097; 1134-1141.

1911. Experiments to determine the toxicity of chromic acid in dilute aqueous solutions when injected intramuscularly and its antidotal value for rattlesnake venom. *University of Pennsylvania Medical Bulletin*, 33: 107.—* Intracellular salins. (In a symposium on the chemistry of the cell.) *Biochemical Bulletin*, 1: 70.

WILLIAM J. GIES, *Secretary*.

BIOCHEMICAL NEWS, NOTES AND COMMENT*

I. GENERAL

Necrology. With deep regret we record the deaths of the colleagues named below :

Dr. K. Polstorff, associate professor of pharmacological chemistry at Göttingen.

Professor J. H. van't Hoff, of the University of Berlin, eminent for his contributions to physical chemistry.

Professor L. P. Kinnicutt, director of the department of chemistry in the Worcester Polytechnic Institute, well-known for his important contributions to sanitary chemistry and sewage disposal.

Dr. Edward B. Voorhees, professor of agriculture at Rutgers College and director of the New Jersey Agricultural Experiment Station, known for his important contributions to agricultural chemistry and agricultural education.

An appreciation of Mrs. Ellen H. Richards, deceased, is published on the opening pages of this issue of the BULLETIN.

The resolutions on page 133, regarding the death of Dr. C. A. Herter, have not hitherto been published.

Officers-elect of biological societies. We give below the names of officers elected at the last annual meetings of leading biological societies :

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE: *President*, Thomas H. Morgan; *Vice President*, Phoebus A. Levene; *Secretary*, George B. Wallace; *Treasurer*, Graham Lusk.

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS: *President*, Lafayette B. Mendel; *Vice President*, A. B. Macallum; *Secretary*, Alfred N. Richards; *Treasurer*, Walter Jones; *Additional members*

*It is intended to make the sections of "*biochemical news, notes and comment*" of the BULLETIN a series of items of historic value and personal interest to biological chemists. The notes comprising the section in this issue have been compiled at random, but succeeding numbers of the BULLETIN will present more systematic compilations of this character. The cooperation of all our colleagues in the execution of this plan is cordially invited.

of the Council, William J. Gies, A. S. Loevenhart, and P. A. Shaffer.

SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS: *President*, John J. Abel; *Secretary*, Reid Hunt; *Treasurer*, A. S. Loevenhart; *Additional members of the Council*, S. J. Meltzer, C. W. Edmunds and Geo. B. Wallace.

AMERICAN PHYSIOLOGICAL SOCIETY: *President*, S. J. Meltzer; *Secretary*, A. J. Carlson; *Treasurer*, W. B. Cannon; *Additional members of the Council*, Joseph Erlanger and Frederic S. Lee.

SECTION OF PHYSIOLOGICAL CHEMISTRY AND PHARMACOLOGY IN THE EIGHTH INTERNATIONAL CONGRESS OF APPLIED CHEMISTRY (September, 1912): Executive Committee—*President*, John J. Abel; *Vice President*, William J. Gies; *Secretary*, John A. Mandel; *Additional members of the Executive Committee*, Reid Hunt and Thomas B. Osborne.

Journalistic. The first number of Volume IX (March issue) of the *Journal of Biological Chemistry* bears the following inscription on the cover:

**THE JOURNAL
OF
BIOLOGICAL CHEMISTRY**

FOUNDED BY CHRISTIAN A. HERTER

EDITED BY

H. D. DAKIN, NEW YORK CITY, LAFAYETTE B. MENDEL, NEW HAVEN, CONN.,
E. K. DUNHAM, NEW YORK CITY, A. N. RICHARDS, PHILADELPHIA, PA.,

WITH THE COLLABORATION OF

(The names of twenty-seven biological chemists are appended to the above.)

Comparing the lists of names of the collaborators of the *Journal of Biological Chemistry*, as printed on the covers of the last issue of Vol. VIII and the first number of Vol. IX, we note the following additions: L. J. Henderson, J. B. Leathes, John A. Mandel, P. A. Shaffer and F. P. Underhill.

In the August issue of the *Journal of Biological Chemistry*, appears an announcement of the Christian A. Herter Memorial Fund. This fund, now amounting to \$40,000 has been entrusted to the care of the directors of the *Journal of Biological Chemistry* under

the provisions of a declaration of trust executed by them. The chief aim of the trust is to further the interests of the *Journal of Biological Chemistry*, an instrument for the development of science created by Christian A. Herter and fostered by him up to the time of his death. In the event that conditions arise removing the need for such a use of the income, provisions are made by which the fund shall continue as a memorial of Professor Herter, and of service to humanity, in the promotion of scientific research. Dr. Simon Flexner, of New York, is the president and Prof. A. N. Richards, of Philadelphia, the secretary of the directorate in charge of the fund.

Prof. Lafayette B. Mendel has resigned the editorship of the Biological Department of *Chemical Abstracts* and Prof. William J. Gies has been elected to succeed him, beginning with the first issue of *Chemical Abstracts* in October.

Honors. Nobel Prizes for 1910 have been awarded in medicine to Dr. Albrecht Kossel, professor of physiology at Heidelberg, and in chemistry to Professor Wallach, the well-known authority on the essential oils and their constituents.

Columbia University has conferred its doctorate of laws on Professor C. F. Chandler, who retires this year from the chair of chemistry after forty-seven years of active service.

The University of Birmingham has conferred the degree of LL.D. upon Prof. R. H. Chittenden.

The doctorate of science has been conferred by Yale University on Prof. Wm. H. Howell, and by Wesleyan University on Dr. F. G. Benedict.

Dr. Harvey W. Wiley was one of the seven eminent scientists and technologists recently honored by the Franklin Institute of Philadelphia with awards of Elliot Cresson medals for "distinguished, leading and directive work" in their respective fields of endeavor. The medals are the highest awards in the gift of the institution.

Dr. Svante Arrhenius was awarded the Willard Gibbs medal of the American Chemical Society for 1911.

Dr. Martin H. Fischer, professor of physiology at the University of Cincinnati, has been awarded the Cartwright prize of \$500, offered by the Alumni Association of the College of Physicians and Surgeons, New York City, for his essay on "Nephritis: An experimental and critical study of its nature, cause and relief."

Dr. Abraham Jacobi was elected president of the American Medical Association, at the last annual meeting.

Dr. Henry Prentiss Armsby, director of the Institute of Animal Nutrition of the Pennsylvania State College, has been elected a member of the Royal Society of Arts of Great Britain.

Dr. Jacques Loeb, of the Rockefeller Institute for Medical Research, has been elected an associate of the Royal Academy of Science at Belgium, in the section of natural science; also an honorary foreign member of the Academy of Medicine of Belgium, a member of the Academy of Sciences in Kharkof, and a correspondent of the Academy of Natural Sciences of Philadelphia.

Various honors have recently been conferred on Dr. Paul Ehrlich, director of the Institute for Experimental Therapeutics at Frankfort. The Emperor of Russia has conferred upon him the Order of St. Anne First Class, with a badge set in diamonds. The King of Spain has bestowed on him the Grand Cross of the Order of Alfonso XII. The German emperor has bestowed on Professor Ehrlich the title of Excellency and has appointed him an active privy councillor. The German physicians who have hitherto received this appointment are Koch, von Behring, von Bergmann and von Leyden. The German Emperor has nominated him a member of the senate of the recently founded Kaiser Wilhelm Society for the Advancement of Science, in which he is now the only representative of medicine. The St. Petersburg Institute of Experimental Therapeutics has elected him an honorary member.

Retirements from Active Service. There have retired from active service Dr. Rudolf Nietzki, professor of chemistry at Basle; Dr. Bernhard Tollens, associate professor of agricultural chemistry at Göttingen; and Professor Rudolph A. Witthaus, professor of chemistry, toxicology and medical jurisprudence at the Cornell University Medical College.

Appointments. The vacancy in the Board of Consulting Scientific Experts to the Secretary of Agriculture, caused by the death of Prof. C. A. Herter, has been filled by the appointment of Prof. Theobald Smith, of Harvard University.

Dr. Thomas Addis will direct the work in clinical chemistry in the Medical Department of Leland Stanford, Jr., University.

Dr. Robert E. Swain has been promoted to a professorship of physiological chemistry at Leland Stanford, Jr., University.

Dr. Adolph I. Ringer has been appointed instructor, and Dr. William C. Rose, assistant instructor, in physiological chemistry at the University of Pennsylvania.

Dr. Henry L. Whittle has been appointed lecturer on physiological chemistry at the College of Physicians and Surgeons in Baltimore.

Dr. Eli K. Marshall, Jr., has been appointed assistant in physiological chemistry at Johns Hopkins University.

Dr. E. C. Franklin, since 1903 professor of organic chemistry at Stanford University, has been appointed professor of chemistry in the hygienic laboratory of the Public Health and Marine-Hospital Service.

Dr. C. H. Neilson, professor of physiological chemistry in St. Louis University School of Medicine, has been made professor of medicine and director of the department.

The title of Dr. Henry C. Sherman, professor of organic analysis at Columbia University, has recently been changed to *professor of food chemistry*.

Dr. William McKim Marriott has been appointed instructor in biological chemistry in Washington University, St. Louis.

Dr. Victor C. Myers has been appointed lecturer in pathological chemistry, and Dr. Morris S. Fine instructor in pathological chemistry, at the New York Post-Graduate Medical School and Hospital.

Lectures and Addresses. Prof. Svante Arrhenius spent several weeks in America last spring, and during his visit he delivered important lectures at several Universities and before various

societies. During his stay in New York City, Prof. Arrhenius lectured at the College of the City of New York, at the Chemists Club and at Columbia University.

Dr. H. W. Wiley delivered the commencement addresses at the University of Maryland and at the Woman's Medical College of Pennsylvania.

Miscellaneous. *Special Research Fund.* The H. F. Kieth Company, of Boston, have given \$5,000 to the Massachusetts Institute of Technology for a research on the decomposition and general wholesomeness of eggs, and for an investigation of the bacterial and chemical contents of the product under varying conditions.

Chemists' Club. The formal dedication of the new Chemists' Club building was celebrated in New York City on March 17 to 19 by a series of scientific meetings and social functions which were greatly enjoyed by a large number of prominent scientific men and members of the American Chemical Society, Society of Chemical Industry, and American Electrochemical Society. At the "extraordinary meeting" of the American Chemical Society on the evening of dedication day, Dr. Jacques Loeb delivered a lecture on "The characteristics of living matter from the physico-chemical point of view."

II. COLUMBIA BIOCHEMICAL ASSOCIATION

Newly elected officers and members of various societies.

Recent elections of members of the Association to office, and to membership, in scientific societies are indicated in the appended summary.

Prof. Isabel Bevier: Elected President of the American Home Economics Association.

Prof. A. D. Selby: Reelected President of the American Phytopathological Society.

Prof. A. N. Richards: Reelected Secretary of the American Society of Biological Chemists.

Prof. Charles R. Stockard: Elected Secretary of the American Society of Naturalists.

Dr. Louis Hussakof: Reelected Secretary of the Biological Section of the New York Academy of Sciences.

Prof. Raymond C. Osburn and Dr. C. Stuart Gager: Elected Fellows of the New York Academy of Sciences.

Prof. L. L. Woodruff: Elected to membership in the American Physiological Society.

Prof. D. D. Whitney and Dr. A. Franklin Shull: Elected to membership in the American Society of Naturalists.

Dr. C. A. Darling: Elected to membership in the Torrey Botanical Club.

Appointments. Dr. A. Franklin Shull, lately assistant in zoology at Columbia University, has been appointed acting assistant professor of zoology at the University of Michigan, where he succeeds Dr. A. S. Pearse who has gone to the University of Manila.

Dr. Herman M. Adler has been appointed instructor in mental diseases at the Harvard Medical School, and will be no longer officially connected with the department of pathology or neuro-pathology. He will retain the position of pathologist at the Danvers State Hospital.

Dr. E. Newton Harvey has been appointed instructor in physiology at Princeton University.

Mr. Jacob Bronfenbrenner, who has recently completed the research requirements for the Ph.D. degree in the department of biological chemistry at Columbia University, has been appointed Fellow in Pathology at the Rockefeller Institute for Medical Research.

Dr. Allan C. Eustis spent the months of March to July inclusive in von Noorden's laboratories as an assistant and also as a special student of pathological nutrition. Dr. Eustis has lately been appointed Assistant in Internal Medicine at Tulane University.

Resignation. Dr. Clinton B. Knapp has lately resigned his Professorship of Bacteriology at the New York College of Pharmacy, owing to the pressure of his general medical practice.

Industrial. Dr. Alfred H. Kropff is President of the Hoffman and Kropff Chemical Co., manufacturers of synthetic organic and analyzed inorganic chemicals, volumetric solutions, etc. This com-

pany, with office and factory at 619 Kent Avenue, Brooklyn, N. Y., was organized about a year ago and is now manufacturing all the finer grades of chemicals employed in advanced instruction and research.

Obituary. We state, with great personal regret, that Dr. W. W. Lesem, a member of the Biochemical Association and a practising physician in New York City, died suddenly on January 10. Dr. Lesem was a man of high ideals, earnest purposes, and sterling scientific abilities. He cooperated with Dr. Gies in the early studies which demonstrated that protagon is a mechanical mixture. About two years ago Dr. Lesem began with Dr. Gies an inquiry into possible chemical functions of the tonsils, but was unable to go forward with it to completion.

We are deeply grieved by the death of Dr. Raymond H. Pond, which occurred suddenly on July 25. At the time of his death Dr. Pond was Plant Pathologist at the Texas Agricultural Experiment Station, and Honorary Third Vice President of the Biochemical Association. He was a charter member of the American Society of Biological Chemists. For several years Dr. Pond was engaged, under Dr. Gies' direction at the New York Botanical Garden, in studies of enzyme action, and for some time prior to his acceptance of the position in Texas he conducted biochemical research under the auspices of the Metropolitan Sewage Commission. Dr. Pond was beloved by a wide circle of friends. His untimely death removes a promising young worker from the small group of American investigators in botanical chemistry.

Secretary's announcements. A number of former workers in the Columbia biochemical laboratories may not have received invitations to join the Biochemical Association. This has been due to the fact that our lists of names and addresses are incomplete. We hope to perfect the lists in the near future and to communicate personally with all who are eligible to membership.

The association will hold its next meeting about the middle of December, when it will be favored with an informal address by Professor R. H. Chittenden.

III. COLUMBIA BIOCHEMICAL DEPARTMENT¹

The Bulletin. The widespread and hearty interest in the Association and in the BULLETIN has been a matter of deep personal and professional gratification to Prof. Gies and all of us now associated with him in the biochemical department.

Personalia. Prof. Gies officially represented Columbia University at the inauguration of his Yale (Ph.D.) classmate, Dr. William A. Granville, as President of Gettysburg College. Prof. Gies is an alumnus, and also one of the elected representatives of the alumni in the Board of Trustees, of that college and in the latter capacity took an active part in the election of Dr. Granville, who, prior to his acceptance of the Gettysburg presidency, was an Instructor in Mathematics in the Sheffield Scientific School.

Prof. Gies has been elected a Scientific Director, and a member of the Board of Managers, of the New York Botanical Garden, to succeed Prof. Charles F. Chandler retired. Prof. Gies has been selected by the Board of Managers to represent the Botanical Garden at the Eighth International Congress of Applied Chemistry. He was recently elected an Associate Member of the American Medical Association.

Prof. Gies and Drs. Eddy and Lothrop were recently elected to membership in Phi Lambda Upsilon.

Dr. Herman O. Mosenthal and Dr. Jacob Rosenbloom have been elected to membership in the Society for Experimental Biology and Medicine.

Dr. Isidor Greenwald has been appointed pathological chemist in the Laboratory of the Montefiore Home.

Dr. Clark has been elected an Associate Editor of the *Bulletin of the Torrey Botanical Club*.

The Journal of the Allied Dental Societies for December, which appeared in January, contains (in the Proceedings of the New

¹Although it will be a policy of the BULLETIN to keep past workers in the Columbia biochemical laboratories intimately acquainted with local affairs, it is also planned to give similar attention, if possible, to all other biochemical laboratories. This plan, like many others connected with the BULLETIN, will be developed in the near future, after more immediate problems have been solved.

York Institute of Stomatology) the following allusion, by President Howe, to an address by Dr. Gies and to work which he is doing with Dr. Lothrop's cooperation, on "the possible relation of saliva to decay of teeth": "We are very much indebted to the speaker this evening for the interest he has taken in our problem of the decay of teeth. Dr. Gies has, from a pure spirit of scientific interest in the study of saliva, taken our problem and made it one of his own, giving us his skill and his time without compensation."

J. Morgan Howe, M.D.S., M.D., Chairman of the Research Committee of the New York Institute of Stomatology, is the author of a notable paper on "The degree of prevention of decay of teeth obtainable by oral hygiene" (*Journal of the Allied Dental Societies*, 1911, vi, p. 236). On page 239 he makes the following remarks:

"The suggestion of Prof. Wm. J. Gies of the Department of Biological Chemistry of Columbia University, that the teeth be washed and brushed with a solution of a vegetable acid, such as vinegar, has had a few favorable reports in cases of great susceptibility to decay and marked inability of patients to keep their own teeth clean, but a sufficient number of trials extending over enough time, have not yet been reported by dentists to give assurance of its results. The reason given by Prof. Gies for the suggestion was that acids are capable of coagulating and breaking up mucin from its adhesions, and that the degree of acidity required would be so slight that no harm to the teeth's structure could result. This would be a radical departure from former ideas, based apparently on the supposition that dental disintegration is the result of the action of acid diffused through the mouth, and that this should be neutralized by alkaline lotions; whereas the worst cases of susceptibility to decay have been found when mouth fluids were markedly alkaline to litmus."

The proposed acid treatment was mentioned favorably last March in a report by the Committee of which Dr. Howe is chairman (*Journal of the Allied Dental Societies*, 1911, vi, p. 54.)

Book Review. Dr. Eddy has lately published a revised edition of his *Experimental Physiology and Anatomy*. This book is an excellent laboratory manual. It is intended for use in secondary schools only, but it is so full of practical suggestions and methods that it will be found very serviceable to any beginner in experimental work in physiology. In his preface Dr. Eddy says that although "the importance of physiology in secondary schools is everywhere

recognized, little attempt has been made to place the subject on an experimental basis. *The recent great advances in physiological chemistry* have directed the attention to the possibilities of the experimental method as a means of investigating the principles of the subject. This book represents a selection of experimental matter which is adapted to the age of elementary students of the subject and which, at the same time, will present the facts of physiology in a concrete form." Seventy-seven exercises are presented, with a special view to supplementing the author's *Text-Book in General Physiology and Anatomy* (1909), and they are so arranged that teachers may select either a minimum or a maximum course. In the revision the changes are due to experience in teaching the first edition and to advances in scientific knowledge. Among these improvements are the introduction of exercises on chemical and physical changes, and on mixtures and solutions; the employment of new reagents to facilitate the application of various chemical tests; the shortening and simplification of experimental procedures; the development of new forms of experiments, etc. The work makes it clear that the principles of nutrition, digestion, absorption, circulation, assimilation, etc., are common to all forms of living matter, and that the most important province of physiology is the explanation of these processes wherever they occur. Human as well as other biological materials have been selected for experimentation, and the *physiology of the cell is made the central point of study*. Only such anatomical dissection is required as is necessary to afford a clear understanding of the action of the parts. The directions are comprehensive yet concise, while the experiments are so planned that they can easily be performed with simple apparatus. The writer has seen numerous statements written by high school teachers in cordial appreciation of the many practical merits of Dr. Eddy's manual.

New Officers. Dr. Welker has been promoted from the grade of Associate to Assistant Professor of Biological Chemistry. (See page 136.)

The following departmental appointments have lately been made: Mr. Edgar Grim Miller, Jr., A.B., assistant in physiological chemistry (Teachers College), vice Miss Mary A. Smeeton, term ex-

pired; Miss Constance Hart, laboratory assistant (Teachers College, summer session), new position; Miss Blanche Harris, laboratory assistant (Teachers College), new position; Mr. Milton V. Miller, A.B., laboratory assistant in organic chemistry (College of Physicians and Surgeons), vice Levering Tyson, term expired.

Doctors of Philosophy. Of the nineteen candidates for the degree of Ph.D., under the Faculty of Pure Science at Columbia University last June, ten had taken majors or minors, or both, in the department of biological chemistry. The names of the candidates and their major and minor subjects are given below:

Name of Candidate	Major	Minor	Minor
G. D. Beal	organic chemistry	chemistry	biological chemistry
G. A. Geiger	organic chemistry	chemistry	biological chemistry
I. Greenwald	biological chemistry	biological chemistry	physiology
R. F. Hare	biological chemistry	biological chemistry	chemistry
E. N. Harvey	zoology	physiology	biological chemistry
M. Heidelberger	organic chemistry	chemistry	biological chemistry
A. McD. McAfee	physical chemistry	organic chemistry	biological chemistry
F. W. Schwartz	physical chemistry	organic chemistry	biological chemistry
A. F. Shull	zoology	biological chemistry	zoology
L. E. Wise	organic chemistry	chemistry	biological chemistry

Summer Session. Courses. During the recent summer session, courses were given in physiological chemistry at the College of Physicians and Surgeons by Prof. Gies and Mr. Clayton S. Smith, and at Teachers College by Prof. Gies and Misses Emily C. Seaman and Constance Hart.

Investigators. Prof. Gies kept the biochemical laboratory at the medical school open daily throughout the past summer. The workers named below conducted investigations during all or part of the vacation:

David Alperin, Jacob Bronfenbrenner, Nellis B. Foster, Simon S. Friedman, William J. Gies, Samuel Gitlow, Mark J. Gottlieb, Isidor Greenwald, Morris Joseph, Max Kahn, David J. Kaliski, John L. Kantor, H. M. Kraus, Alfred P. Lothrop, Chester A. Mathewson, Herman O. Mosenthal, Reuben Ottenberg, Oscar M. Schloss, Emily C. Seaman, Clayton S. Smith, William Weinberger, Julius W. Weinstein, Charles Weisman, William H. Welker, Harry Wessler.

EDITORIALS

This journal will be devoted to the promotion of biochemical research and to the extension of biochemical knowledge. The BIOCHEMICAL BULLETIN will be issued quarterly—in March, June, September and December. It is addressed directly to the expanding circle of students and investigators whose collective ability and industry have given the Columbia biochemical laboratories an honorable history of endeavor and usefulness.

Purpose and scope
of the
Biochemical Bulletin

The BULLETIN will perform functions similar to those of the conventional alumni journal. It will aim to keep all the members of the Columbia biochemical family in touch with the old laboratory homes. It will seek to maintain cordial personal and professional sympathy between the resident workers and those who have gone to other laboratories.

In pursuance, however, of the primary purpose to make the BULLETIN a broadly effective agent for the advancement of biological chemistry, subjects of general as well as local interest will be presented in every issue. Biological chemists everywhere are cordially invited to send us communications of any character whatever that will increase the value and add to the interest of the BULLETIN. Original contributions to research, preliminary reports of investigations, abstracts of papers, addresses, reviews, descriptions of new methods and apparatus, practical suggestions to teachers, biographical notes, historical summaries, bibliographies, quotations, news items, personalia, and views on current events in chemical biology, are especially solicited.

This journalistic mutant begins its career under auspicious circumstances and in a favorable environment. That it will grow and be abundantly useful is the hope and belief of all who now formally offer it their encouragement and support.

If the BIOCHEMICAL BULLETIN becomes as useful in its own field as the *Johns Hopkins Hospital Bulletin* has been and continues to be in Medicine, our editorial efforts will be amply repaid.

Unforeseen difficulties have greatly delayed the publication of this the initial number of the BULLETIN. This issue contains much of the material in our hands on the first day of September. For the sake of convenience this number of the BULLETIN is dated September, 1911. The December number is in press and will be ready for distribution before Christmas.

With the signing of the agreement between Columbia University and the Presbyterian Hospital, medical education in New York enters upon a new phase. In direct consequence of this union **Columbia University and the Presbyterian Hospital** it will become possible in the future for the clinical teaching of medicine to reach the scientific and pedagogic efficiency long since achieved in the laboratory courses of the first two years of the medical curriculum. Despite all the evident advantages of the relationship, it is improbable that the accomplishment of this long desired opportunity will result in any immediate and remarkable change in medical teaching, for similar relations between hospitals and medical schools have existed elsewhere and the graduates of the College of Physicians and Surgeons have not suffered in competition with those from such schools. The new arrangement will simplify the question of obtaining teaching material, and will concentrate instruction in one place. Greater opportunities, however, only bring greater responsibilities. An organic, not merely a physical, union must be the final goal. A new generation of medical and surgical teachers will have to be developed; a new generation of hospital managers may come, before the full realization of this great opportunity is possible. A medical millenium, though announced by the sanguine, is hardly at hand. Nor will the new arrangement prevent Columbia from entering into relations with other hospitals, of a different and somewhat less intimate nature. With the increasing number of students which may shortly be expected, wider clinical facilities will be required, and no doubt some of the tentative agreements now in force, permitting the admission of students to the hospitals of New York, will be continued and extended. The pressing need of opportunities for postgraduate instruction, which is entirely different from the teaching of undergraduates, also demands that the university

keep in touch with a large clinical material, more varied and more extensive than any one hospital can offer.

The situation is remarkable in that it shows that managers of hospitals have come to a realization that the plant and money of which they are trustees should have a wider application than has heretofore been considered as either wise or necessary. The act of the managers of the Presbyterian Hospital in voluntarily offering a medical school the facilities of its wards for student teaching is a recognition by men of affairs that the present situation has become educationally intolerable, and so will be of the greatest value in influencing other boards to an appreciation of the broader aspects of their responsibilities to the patients under their care. (*Columbia University Quarterly*, 1911, xiii, p. 317.)

The Trustees of Columbia University have performed a graceful and appropriate act in designating the chief professorship of physiology the Dalton professorship, in honor of John Call Dalton,

The Dalton Professorship of Physiology M.D. Dr. Dalton entered the College of Physicians and Surgeons as a lecturer at the session of 1854-55, and in the following year became the professor of physiology and microscopic anatomy. A graduate in arts and medicine of Harvard, he had obtained his special training in physiology under Claude Bernard in Paris, where he became imbued with the spirit of the experimental method. He was in point of time the first experimental physiologist in America. He introduced into this country and into the College of Physicians and Surgeons the idea that physiology is an experimental science, and he was the first American teacher of physiology who taught by demonstration as well as by word of mouth. His demonstrative lectures produced a furore of enthusiasm among the students of the college, who still testify to the power and charm of his instruction and his personality, and the singular grasp and lucidity of the text-book of which he was the author. His book easily took the foremost place among American text-books of physiology of its day. Dalton was an early and shining example of the man of medicine who foregoes the activities of practice to devote himself to experimental research and practical teaching. He pointed out the direction in which American

physiological teaching and research were to proceed. It is upon the basis of his pioneer work that the present laboratory instruction of all students of physiology has been developed at Columbia. He suggested the creation of the Swift fund for supplying the department of physiology with apparatus. For five years he was president of the College of Physicians and Surgeons. During this period momentous changes occurred in the college. These included its establishment at its present site and a great extension of its work in all directions, especially along the lines of the clinic and the laboratory. It is eminently fitting that the name of Dalton should be preserved in the title of the chair to which he gave distinguished service, and in the school in the development of which for thirty-five years he was a potent influence. (*Columbia University Quarterly*, 1911, xiii, p. 319.) Dr. Frederic S. Lee is the first Dalton Professor of Physiology.

Under a perversion of the intent of the patent and trade-mark laws of the United States a system of perpetual patent has grown up which is known as the "proprietary medicine system."

Scientific names vs. Trade designations	The United States laws permit the protection of medicines either by patent or by trade-mark in the same way that other commodities are protected. Some countries grant patents on the process only—that is, on the method by which a new medicinal substance is made—but do not grant patents on the substance itself. The United States, however, grants "product" as well as "process" patents. In addition to the product or process patent, the manufacturer can also register a coined name as a trade-mark, which is primarily intended to identify his brand of a substance in the trade. The patent is granted for a period of seventeen years. A trade-mark is practically perpetual. Medicine promoters subverting the original intent of the trade-mark, have used and advertised the trade-marked name as the only name for their particular product. The trade-marked names "chloretone" (chlorbutanol) and "urotropine" (hexamethylenamin) and those applied to the numerous mixtures containing phenolphthalein are good illustrations. The scientific name is used in patenting the medicinal substance, but it is usually sold
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under the non-descriptive, trade-marked name. The name used in the patent becomes free with the expiration of the patent, but the trade-marked name remains the manufacturer's forever—at least according to his claims. In the case of medicines used by physicians, the physician has got into the habit of prescribing them under the trade-marked name; and even after the patent runs out, and the product itself has become common property, the physician who continues to use the trade-marked name continues to play into the hands of the one who patented the product. Thus, the drug first known as phenacetin, but which is designated officially as acetphenetidin, was originally patented. The patent has expired, but the word phenacetin has become so fixed with the average physician that he continues to use it. No one can put out the product under the name phenacetin, and when a physician prescribes phenacetin the druggist is supposed to supply phenacetin. The same is true of many other substances, as for instance, sulphonal (sulphonmethane). The patent on that drug expired long ago, but many physicians continue to use this term—sulphonal—and each time they do it they are helping the sales of those who have reaped the harvest from the protection already given them by the patent.

The abuses of our patent and trade-mark laws may take a number of forms, owing to their imperfections and lax enforcement. Because of an unwillingness on the part of one manufacturer to attack the validity of a patent owned by a competitor, many patents are issued and held on products which present nothing new, and hence protection is given where none is deserved. The following are illustrations of these conditions:

Acetylsalicylic acid (aspirin) was discovered by Kraut in 1868. In practically all European countries this product is not protected and anyone may make and sell it. In this country the product is protected by a patent, and a permanent injunction has been granted by a circuit court to prevent infringement because the patent contains a process for the purification of the drug, an improvement which is not described by Kraut. The holders of the patent claim the exclusive right to make the product in this country—and they sell it at an exorbitant price—while in the country in which it was originally made it is free. *Bismuth betanaphtholate* is pro-

tected by a United States patent and the firm which holds this patent has offered it for sale under the proprietary non-descriptive name "orphol." On examination in the chemical laboratory of the American Medical Association, orphol was found not to be bismuth betanaphtholate at all (*Journal of the American Medical Association*, Dec. 18, 1909, p. 2110). A suit for an infringement of this patent would no doubt have been defeated in court; yet the manufacturer of a superior product, sold under its chemical name, discontinued the sale of his product because the patentee protested against it.

Such abuses of the patent and trade-mark laws by commercial interests may have to be corrected through the courts or by an amendment of the laws. The Committee on Patents and Trade-Marks of the American Medical Association has recommended action by the Association (*Journal of the American Medical Association*, June 19, 1909, p. 2063). *However, it is within the power of physicians to improve these conditions by giving preference, as far as possible, to the chemical or scientific name of an article over the proprietary name.* Physicians should familiarize themselves with such pharmacopeial titles as acetphenetidin ("phenacetin"), hexamethylenamin ("aminoform," "formin," "urotropin," etc.) and sulphonmethane ("sulphonal"), and use them in preference to the proprietary names for these substances. The Council on Pharmacy and Chemistry of the American Medical Association provides scientific names as synonyms for unofficial substances manufactured by more than one firm, and if the prescriber prefers any particular product he may append the firm's initials to the scientific name of the article.

The Council on Pharmacy and Chemistry of the American Medical Association was originally created to protect the medical profession against fraudulent proprietary medicines. After giving the subject full consideration, the Council determined to announce those among the proprietaries that were worthy of recognition by the profession, rather than to condemn those which were more or less fraudulent and not worthy of recognition. In line with this proposition it was decided to publish a book to contain a list of the articles considered worthy, with such information regarding them

as would be of value to the profession; this book to be kept up to date by supplements and by annual revision. The book is known as "New and Nonofficial Remedies," or abbreviated "N. N. R."

How may teachers in medical schools help? By pointing out how progress in therapeutics is retarded and how the health or lives of patients may actually be jeopardized by the use of unscientific names for medicinal substances. For instance, dangerous doses of acetanilid repeatedly have been prescribed by combining in one prescription two or more acetanilid preparations, the names of which gave no indication of their nature. It might also be pointed out that the superiority claimed for proprietary protected products does not, in fact, exist. Thus, Professor Base reported (*Journal of the American Medical Association*, Oct. 12, 1907, p. 1295) that the non-proprietary brands of hexamethylenamin were of good quality, notwithstanding the contrary assertion of the promoters of certain proprietary brands of this drug.

The medical student should be made familiar with the scientific names of drugs. *This can be accomplished by requiring a better knowledge of chemistry and by insisting on the intelligent use of chemical and pharmaceutical names by all teachers in medical schools.*

If one were entirely dependent on the advertisements of drug houses for his information concerning iron therapy he would form the following conclusions:

Fallacies in

Iron Therapy

1. That inorganic iron is absorbed with difficulty and is prone to cause gastric indigestion.
2. That organic iron is more easily absorbed, that it is immediately available for use in enriching the blood; that therefore it is vastly superior to inorganic iron for therapeutic purposes.

These conclusions, however, are not founded on fact. It is at present believed by most authorities that a great excess of iron is maintained by the body in the liver, marrow, spleen and other "storehouses"; that all iron ingested, whether inorganic or organic, is first carried into these depots and there converted into organic iron. It is generally admitted by pharmacologists that the inorganic iron salts are as readily absorbed and assimilated as the or-

ganic compounds of iron. Furthermore, it has been the experience of clinicians that in the treatment of anemias the inorganic iron preparations have not been surpassed, perhaps not equaled, by the numerous organic compounds on the market. It is highly significant that our leading internists remain loyal to such preparations as the tincture of ferric chlorid, reduced iron and Bland's pill in preference to the organic preparations.

Would not iron therapy be greatly simplified and improved by inculcating in the student mind a more critical attitude toward the fanciful claims of manufacturers for their numerous organic iron compounds?

We hear much now-a-days, among biologists and a few chemists, about purin "*bodies*" and acetone "*bodies*," and many other "*bodies*," when certain groups of substances are discussed. What is the difference between a substance and a body?

Shall substances be called bodies? Both have weight and both occupy space. In the case of a body, however, the *shape* is a fundamental feature, whereas with a substance such is not the case. A body has a discernible and particular shape. Any given amount of acetone has a definite mass, but what is the *shape* of acetone or of any of the substances related to it?

On another page (57) we give a brief account of the organization, over a year ago, of the Columbia University Biochemical Association. The Columbia "biochemical family" is animated by the amiable desire to work unitedly for the advancement of biological chemistry. It is proceeding cheerfully and optimistically in this direction with its plans—among them the publication of this BULLETIN. We hope to have and to hold the approval and good will of all who cherish the advancement of science.

It is our present intention to publish, in an early issue of the BULLETIN, a full list of the members of the Biochemical Association. The former workers in the Columbia biochemical laboratories are so numerous that, thus far, we have found it impossible to get into communication with all of them.

The portrait of Mrs. Ellen H. Richards, which accompanies Miss Seaman's appreciation on the opening pages of this issue of the BULLETIN, appeared in the February number of the *Journal of Home Economics*. We are greatly indebted to Mrs. Abel for permission to reproduce the portrait and for the use of the plate for that purpose.

The American Chemical Society is one of the largest organizations of scientists in the world, having now over 5,600 members, including nearly all the prominent chemists in America, and many foreign chemists as well. The large number of new members that have joined in the last twelve months is in itself a guarantee of the Society and its publications. Chemical knowledge is fostered in every conceivable way and the members are offered every possible opportunity to keep abreast of the advancement of chemical science and industry.

**The American
Chemical Society**

Membership is open to all reputable persons interested in chemistry. There is no initiation fee.

Annual payment of \$10 (plus \$2.50 postage to foreign countries) entitles the member to the three Journals published by the Society for the year, beginning with January—*Chemical Abstracts*, the *Journal of the American Chemical Society* and the *Journal of Industrial and Engineering Chemistry*. Members also have full privileges of the Local Sections.

Address all inquiries for application blanks, subscriptions, etc., to Charles L. Parsons, *Secretary*, New Hampshire College, Durham, New Hampshire.

Chemical Abstracts is in its fifth volume. It is issued twice a month by the American Chemical Society, and gives carefully classified abstracts of all important new work in chemistry, both pure and applied. Over 400 Journals are regularly abstracted on its pages. Volume IV contains 13,006 abstracts, covering 3,314 pages. *Chemical Abstracts* has met with marked success and is rapidly gaining subscribers, as it occupies for the English reading world the place which *Chemisches Zentralblatt* holds for the German.

Chemical Abstracts

The Editor of *Chemical Abstracts* is assisted by two Associate Editors, a staff of Assistant Editors and over three hundred Abstractors. The abstracts are conveniently divided into groups covering all branches of chemical science and industry with copious cross references. The Department of Biological Chemistry, under the editorship of Prof. Lafayette B. Mendel, has been particularly successful and serviceable. Brief abstracts of American, English, French, and German chemical patents are included; also, titles and prices of new books.

Chemical Abstracts is issued on the 10th and 20th of each month. Although but four volumes have been published, it already has by far the largest circulation of any scientific abstract journal in the world. The subscription price, to non-members of the American Chemical Society, is \$6.00 per year. Foreign postage, \$1.00.

Science advances because it is never sure of anything. *Duclaux.*

In experimental science it is always a mistake not to doubt when facts do not compel affirmation. *Pasteur.*

Enzymes

Distinction is the consequence, never the object, of a great mind. *Allston.*

The same force which shapes the raindrop or the molten mass of a planet (surface tension) is an all-important factor in the causation of vital phenomena. *Macallum.*

The way to truth leads through error. All progress consists in showing that the past truths were errors, or at best half-truths. That does not make them superfluous; they constitute the stages by which the human mind ascends its precipitous path. *Paulsen.*

Work perseveringly. Work can be made into a pleasure, and alone is profitable to a man, to his country, to the world. Whatever career you may embrace, look up to an exalted goal. Worship great men and great things. *Pasteur.*

It is almost a truism to assert that the progress of knowledge mainly depends on the invention of new experimental methods, or the perfection of old ones. Science owes a great deal to the reasoning power of the thinker, and to the acumen of the guesser, but both are alike futile until facts are accurately determined. *Halliburton.*

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(*Abbreviations:* C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

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51. ELEMENTARY ORGANIC CHEMISTRY. Introductory to courses 101, 102 and 104. (*Required of first year students of medicine.*) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, and Mr. Smith.

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This course is designated "S—H. A. 25" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies and Miss Seaman.

104. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R, 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, Dr. Clark, and Messrs. Smith and Rose.

This course is designated "S—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Mr. Smith.

201-202. CHEMISTRY OF NUTRITION. (School of Pharmacy. *Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

203-204. GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* L, 1 hr. Lw, 4 hr. Dr. Eddy.

Courses in Nutrition (continued)

205-206. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (Teachers College, School of Household Arts.) L, 1 hr. Lw, 5 hr. Prof. Gies and Miss Seaman. (This course is designated "H. A. 125" in the Teachers College Announcement.)

207-208. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS. L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Lothrop.

209-210. NUTRITION IN HEALTH AND DISEASE. L, 2 hr. Prof. Gies.

211-212. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies, Dr. Clark and Mr. Rose.

213-214. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies.

215-216. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Welker, Drs. Lothrop and Clark and Mr. Rose.

TOXICOLOGY

217-218. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. Lw, 6 hr. Prof. Gies.

BOTANY

219-220. CHEMICAL PHYSIOLOGY OF PLANTS. (New York Botanical Garden.) L, 1 hr. Lw, 5 hr. Prof. Gies and Dr. Clark.

BACTERIOLOGY

221-222. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Clark.

SANITATION

105. SANITARY CHEMISTRY. (Teachers College, School of Household Arts.) L, 1 hr. Lw, 3 hr. Professor Gies, Miss Seaman and Dr. Clark. (This course is designated "H. A. 26, a" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301-302. BIOCHEMICAL SEMINAR. 1 hr. Prof. Gies.

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Biochemical research may be conducted, by advanced workers, independently or under guidance.

BIOCHEMICAL LIBRARY

Prof. Gies's library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons, and is accessible, by appointment, to all workers in the Department.

LABORATORIES FOR ADVANCED WORK IN BIOLOGICAL CHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College and the New York Botanical Garden.

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The Biochemical Association holds quarterly scientific meetings, which are open to all students in the University.

Biochemical Bulletin

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THE BIOCHEMICAL BULLETIN

The BIOCHEMICAL BULLETIN is a quarterly journal of biochemical notes and news. It publishes results of original investigations in chemical biology and presents miscellaneous items of personal and professional interest to biological chemists.

Biological chemists everywhere are cordially invited to forward contributions of any character whatever that will increase the value and add to the interest of the BULLETIN. Original contributions to research, preliminary reports of investigations, abstracts of papers, addresses, reviews, descriptions of new methods and apparatus, practical suggestions to teachers, biographical notes, historical summaries, bibliographies, quotations, news items, personalia, and views on current events in chemical biology, are especially solicited.

The BULLETIN will present as much biochemical substance of as great variety and value, in as little space and at as small an annual subscription price, as possible. Contributors are accordingly requested to keep their papers within the bounds of 15 printed pages, if possible, and to *restrict them to 10 pages or less*, if it can be done satisfactorily. Recrystallize the products repeatedly, reject the mother liquors and send the BIOCHEMICAL BULLETIN "preparations of *tested purity*"!

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Two young men of exceptional training in chemistry and biological chemistry, in this country and abroad, seek University positions—one in organic chemistry, one in biological chemistry.

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New Biochemical Books

A Text-book of Physiological Chemistry. By Olof Hammarsten. Authorized translation from the author's enlarged and revised seventh German edition, by John A. Mandel. *Sixth American edition.* 8vo, viii + 964 pages. Cloth, \$4.00 net. John Wiley & Sons, New York; Chapman and Hall (Limited), London. See Lafayette B. Mendel's review, *Science*, 1911, xxxiv, p. 883 (Dec. 22).

Nephritis. *An experimental and critical study of its nature, cause and the principles of its relief.* By Martin H. Fischer. The 1911 Cartwright Prize Essay of the Alumni Association of the Columbia Medical School. 12mo, ix + 203 pages, illustrated. Cloth, \$2.50 net. John Wiley & Sons, New York; Chapman and Hall (Limited), London.

Immune Sera. *A concise exposition of the main facts and theories of infection and immunity.* By Charles F. Bolduan. 12mo, xi + 226 pages, illustrated. Cloth, \$1.50 net. John Wiley & Sons, New York; Chapman and Hall (Limited), London. See review: This issue of the *BIOCHEMICAL BULLETIN*, page 358.

Biochemical Bulletin. Papers for the March Issue

Papers for the March issue of the *BULLETIN* have been received from the contributors named below:

George D. Beal and George A. Geiger, Sidney Born, William J. Gies, Max Kahn, Max Kahn and Jacob Rosenbloom, John A. Mandel, Albert P. Mathews, Anton R. Rose, Fred J. Seaver and Ernest D. Clark, Jacob Shulansky, Ernest E. Smith, Charles Weisman, William H. Welker, and Lorande Loss Woodruff.

BIOCHEMICAL BULLETIN

VOLUME I

DECEMBER, 1911

No. 2

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GARDEN.

A REVIEW OF THE HISTORY OF BENCE JONES PROTEIN AND MULTIPLE MYELOMA

JACOB ROSENBLOOM

(Laboratory of Biological Chemistry of Columbia University, at the College of
Physicians and Surgeons, New York)

I. INTRODUCTION

In connection with an experimental study of the origin of Bence Jones protein, I had occasion to prepare a review of our knowledge of this important substance.¹ That review is included in the present paper.

It was my intention, before beginning the experimental investigation of Bence Jones protein, to make a list of all the known cases of myeloma and Bence Jones proteinuria with the characteristics of each, but this was found to have been done by Anders and Boston (1), who recorded cases prior to 1903 and gave a report of three new cases: by Weber (2), who recorded 28 cases prior to 1904 and gave the history of ten more cases; by Moffat (3), who recorded 39 cases prior to 1905; and by Permin (4) who recorded 40 cases prior to 1907. Decastello (5), in a recent paper, described two more cases and made an analysis of those previously recorded.

In 1847 Bence Jones (6) presented before the Royal Society of London, a paper "On a New Substance Occurring in the Urine of a Patient with *Mollities ossium*," in which he described, for the first time, the substance since known as the Bence Jones protein. Later (7) he described several properties of this substance, and gave the results of a study of it in his report on *Mollities ossium*. The

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Bence Jones protein was rediscovered and described by Kühne (8) in 1869. It has since been the subject of many investigations, especially by Matthes (9), Ellinger (10), Magnus-Levy (11), Jochman and Schumm (12), Bradshaw (13), Moffat (14) and Simon (15). Magnus-Levy (11), and also Grutternick and de Graaf (27), have succeeded in obtaining Bence Jones protein in crystalline form.

Does Bence Jones protein occur in bone? Ellinger (10) succeeded in obtaining Bence Jones protein in small amounts from diseased bone marrow. Hopkins and Savory (16) could not find the substance in the bone marrow from the cases they studied. Virchow (17) found it in the bone marrow in cases of osteomalacia, so-called. Barr (18) could not find in the bone marrow substance of bone tumor, any trace of Bence Jones protein or of enzymes. Wood (19) claims to have separated Bence Jones protein from a portion of bone affected by multiple myeloma, but could not obtain it from the bone marrow in any other portion of the body of the patient. Askanazy (20) was able to demonstrate its presence in the bone marrow of a case of multiple myeloma but was unable to find it in blood from this patient. Löwy (21) could not detect a trace of Bence Jones protein in the marrow of the affected ribs and humerus of Kalischer's case. Weber (22), however, was able to prove the presence of a substance giving reactions similar to those of Bence Jones protein, in the vertebrae and ends of the femur in a case of multiple myeloma, but he could not detect this substance in any other tissue or organ. Bruce, Lund, and Whitcomb (23) found, in a case of multiple myeloma, that the fluid obtained from an affected bone, after sawing through it, gave the reactions of the Bence Jones protein. Ribbinik (24) could not find Bence Jones protein in the bone marrow substance of the case studied by him. Fleischer (25), however, has found a substance giving the reactions of Bence Jones protein in normal bone marrow. In a case of Weber's (2), microscopic section of some of the organs and the new growth in multiple myeloma showed the presence of a homogeneous hyalin substance which he thought might possibly be Bence Jones protein. Bradshaw and Warrington (26), in an analysis of a rib affected with multiple myeloma, found the relation of organic and inorganic substances to be practically normal.

Digestive products of Bence Jones protein. Moitessier (28) on subjecting Bence Jones protein to gastric digestion obtained metaprotein, primary proteoses (except heteroproteose), secondary proteoses and peptone. After peptic digestion of Bence Jones protein, Simon (15) could not detect primary proteoses among the products formed, but found deuteroproteose "B" (Pick) and peptone "A" (Pick). Coriat (36) found Bence Jones protein digestible in artificial pancreatic juice.

General chemical nature of Bence Jones protein. Magnus-Levy (11) published results of a study of the digestive products of Bence Jones protein, its reaction and content of amid, diamino and monoamino nitrogen. Huppert (29) recorded results of various elementary analyses that have been made of Bence Jones protein. Abderhalden and Rostoski (30) made an analysis of Bence Jones protein to determine the amounts of the various amino acids contained. Hopkins and Savory (16) found that Bence Jones protein yields all the amino acids characteristic of typical protein, and that it contains a large proportion of aromatic radicals. Reach (31) gave the results of an analysis of Bence Jones protein in terms of its nitrogen partition. Gross and Allard (37) administered 10 grams of Bence Jones protein to an alkaptonuric subject, with otherwise unaltered diet. There was a large increase in the output of homogentisic acid, which suggested to Gross and Allard that Bence Jones protein is rich in aromatic radicals.

Bence Jones protein in urine. Zuelzer (32) obtained Bence Jones protein in the urine of dogs poisoned with pyrocin (monocetyl-phenylhydrazine), a strong hemolytic agent. Stokvis (33) found Bence Jones protein in the urine of dogs after its intravenous or rectal injection. Matthes (9) also found it in the urine of a dog after the subcutaneous injection of Bence Jones protein. Ellinger (10) introduced 5 grams of Bence Jones protein intravenously into a dog, but the urine yielded no precipitate with $(\text{NH}_4)_2\text{SO}_4$, although the liquid gave a strong biuret reaction suggestive of peptone (Kühne), which may have been derived from the injected material. Rosenbloom (R) has studied a case in which the Bence Jones protein was spontaneously precipitated from the urine.

Allard and Weber (34) found that the Röntgen ray treatment

of the bone tumor had no effect on the urinary output of Bence Jones protein. Voit and Salvendi (35) reported a case in which diet appeared to modify the elimination of Bence Jones protein, but Weber observed that changes of diet had no influence on its elimination in his case of multiple myeloma. Hopkins and Savory (16) found that the amount of excreted Bence Jones protein was proportional to the extent of metabolism rather than to any other factor.

Bence Jones protein in blood and lymph. Ribbinik (24) and Askanazy (20) could not find Bence Jones protein in the blood of patients with multiple myeloma. Ellinger (10) obtained it from ascitic fluid. Coriat (36) found Bence Jones protein in a pleural effusion from a patient suffering from multiple neuritis associated with extreme tenderness of the ribs, although the protein was absent from the urine. Rostoski (38) found that the method of "precipitin" detection fails to distinguish Bence Jones protein from various proteins of human origin. Donati and Satta (39) have shown that serum albumin, serum globulin, edestin, egg albumin, milk, and milk serum, inhibit the hemolytic action of sodium glycocholate and sodium oleate, whereas ovo-albumin, Bence Jones protein, and casein, accelerate the hemolysis. Borchart and Lippmann (40) found that after feeding Bence Jones protein to fasting dogs, it could be detected in serum from the animals by the precipitin test and in samples of their blood by chemical tests.

II. PROTEOSURIA AND BENCE JONES PROTEIN

Proteoses have been found in the urine under many conditions, usually in minute quantities and as temporary constituents of the urine during the course of specific fevers, inflammatory processes, and other diseases. The urinary proteoses present different characteristics from those of Bence Jones protein, however, and must be sharply distinguished from the latter. Among the most prominent of these differences between Bence Jones protein and ordinary proteoses, the following may be indicated in terms of Bence Jones protein:

1. Soluble in water (different from heteroproteose).
2. Coagulated at low temperatures (unlike other proteoses col-

lectively), though elastoses are precipitated by heating their aqueous solutions but *redissolve* as the temperature falls.

3. Convertible into metaproteins (unlike other proteoses collectively).

4. Digested by pepsin-HCl, yields protoproteose (unlike all proteoses).

5. Not acted upon by erepsin (different from proteoses).

6. Excreted in larger proportions than the proteoses.

7. Does not dialyze through parchment paper (different from all soluble proteoses).

8. Not precipitated from saline solution by dialysis (different from several proteoses).

III. THEORIES REGARDING THE ORIGIN AND THE NATURE OF BENCE JONES PROTEIN

Proteose relationships. Kühne (8) believed that Bence Jones protein is closely related to heteroproteose on account of the fact that the pure substance, after its precipitation from its solution by heating, redissolves with further elevation of the temperature. Huppert (29) also thinks the protein is a heteroproteose. Dechaunne (41) considers it a mixture of at least three proteins or groups of proteins, probably proto- and dysproteoses, and a substance like heteroproteose. Kühne and Chittenden (43) found that, on the basis of elementary composition, Bence Jones protein resembled heteroglobulose. Neumeister (42) showed that Bence Jones protein is not heteroproteose. He did not believe that there is any relation between digestive conditions and the presence of this substance in the urine. He thought rather, that it is a substance of a peculiar kind and quite unlike any other that had hitherto been described. Matthes (9), was of the same opinion as Neumeister.

Possible derivation from blood proteins. Simon (15) thinks Bence Jones protein is formed from the serum globulins, perhaps by an enzymotic action of the tumor cells, and that once produced, it is rapidly excreted by the kidneys as are all foreign proteins. Kühne and Chittenden (43) suggest that it may arise from serum globulin. Coriat (36) also thinks it might be formed from

serum globulin. He supposes it is produced by the digestive action of leucocytes or bacteria, or, more particularly, by the enzymotic action of plasma cells in the bone marrow. Moitessier (21) believed that Bence Jones protein is derived from blood proteins under the influence of new bone growth. Lindeman (49) concluded that, while Bence Jones protein cannot be put in any present group of proteins, it is nearest in relationships to the true albumins. Abderhalden (50) stated that, judging from its yield of amino acids, Bence Jones protein does not correspond to either of the two serum proteins, but may be considered a tissue albumin, which without being broken down or changed into one of the serum albumins, is transmitted to the blood and is then probably eliminated as an albumin foreign to the blood. Schulz (65) suggested that the Bence Jones protein might prove to be related to globin.

Origin from bone. Donetti (44) believes that Bence Jones protein results from some loss of function of the bone marrow, owing to the destruction of the latter. Hopkins and Savory (16) concluded that it is formed by processes that indicate interruptions in the normal autolytic processes in tissues due to toxin from the growth. They also suppose that the loss of some normal function of the bone marrow may give rise to Bence Jones protein. Weber and Hutchinson (22) concluded that Bence Jones protein is formed from granules in the myelomatous cells. Virchow (17) believed the substance resulted from degenerative changes in protein occurring in sarcomata. Weber (22) also thought it may be due to an abnormal metabolic or degenerative process in the myelocytes, or in the tumor cells derived from the myelocytes or their predecessors. Von Rustizky (45) likewise considered that the substance is produced in connection with new bone growth. Williams (51) thought that Bence Jones protein may represent modified glycoproteins from disorganized bones and tendons. He accounts for the variable properties of Bence Jones protein products by assuming that the glycoproteins are more or less broken up and then excreted in different degrees of chemical decomposition according to the stage of the disease. In a recent paper Weber and Ledgingham (46) suggested, from the histological evidence in the case of multiple myeloma studied by them, that the cytoplasmic residues of karyolyzed plasma cells may be the source of Bence Jones protein.

Ottenberg and Gies (52) have found in this laboratory that crude elastose, after its subcutaneous or intraperitoneal injection, can readily be detected in the urine by the heat precipitation test. Since Bence Jones protein has various properties in common with elastoses, Ottenberg and Gies suggested that ossealbumoid (bone elastin?) might be the forerunner of Bence Jones protein.

Several years ago, working under the direction of Prof. Gies, I (53) endeavored to determine whether ossealbumoid might be so acted upon by the enzymes present in cells of myelomatous growths as to give rise to a substance having the properties of Bence Jones protein. The results of that investigation suggested that Bence Jones protein *may be* formed from ossealbumoid by the action of enzymes present in bone marrow.

A product of abnormal metabolism. Senator (47) inclined to the view that the Bence Jones protein represents a product of the abnormal metabolism of food protein. Magnus-Levy (11) also thought it was formed from food proteins as a result of altered protein metabolism. As much as 30 to 70 grams of Bence Jones protein may be excreted per day, whereas the total amount of protein in all the tumor tissue seldom exceeds, or indeed equals, this quantity. Magnus-Levy considers it impossible for so much urinary protein to arise from so little tumor tissue. Rostoski (48) advanced the same view. Hopkins and Savory (16) concluded, from studies of metabolism and effects of diet, that Bence Jones protein is a product of endogenous metabolism.

It is possible that multiple myeloma is due to a specific bacillus, which by the action of its toxins so alters the normal changes occurring in bone marrow as to produce this substance from the tissue protein. This idea is strengthened by the analogy Weber has drawn between the characteristics of multiple myeloma and mycosis fungoides, which is thought by some to belong to the group of infective granulomata. This view is strengthened by the fact that in the case studied by Weber and Ledgingham (46) the growth consisted of plasma cells. The sarcoma-like tumors of the skin, known as mycosis fungoides, have been found to be plasmomata. Another idea that might be held as to its mode of formation is the following: Possibly the columnar epithelium that lines the ali-

mentary canal is diseased. The agent that converts the digestive products may therefore fail to function. In consequence, the incompletely synthesized products are taken into the blood stream and then eliminated as matter foreign to it. This theory seems improbable on account of the fact that in cholera, when marked changes are present in the columnar epithelium, Bence Jones protein is not excreted, although, of course, in cholera the said changes may be insufficient in kind or degree to produce the result. Abderhalden and Rostoski (30) have shown that Bence Jones protein yields a "precipitin" which is active with human serum. It must therefore represent assimilated material and cannot be an exogenous product derived directly from intestinal processes.

IV. BENCE JONES PROTEIN AND MULTIPLE MYELOMA—MYELOPATHIC PROTEOSURIA (KAHLER'S DISEASE)

In 1889 Kahler (54) and Huppert (55) reported a case of multiple myeloma from clinical and chemical standpoints, respectively, and in 1897 Bozzola (56) reported a case under the title of "*Sulla Malattia di Kahler*" thus recognizing Kahler as the first to show the relationship between proteosuria (so-called) and primary bone disease. These lesions, however, were classified in 1873 as myeloma by Von Rustizky (45).

Careful study of cases of Bence Jones proteinuria shows that there must certainly be some relation between the excretion of Bence Jones protein and diseased conditions of the bones. Although we cannot say that Bence Jones protein is peculiar to the growth known as multiple myeloma,² it is certain that Bence Jones protein is present in the urine in 80 per cent. of the cases exhibiting this condition. In cases of excretion of Bence Jones protein unaccompanied by multiple myeloma, disease of the blood-forming organs or of bone has been present.

Weinberger (57) found Bence Jones protein in urine from a

² Various names applied to multiple myeloma: Myeloma multiplex (Rustizky), sarcoma multiplex ossium (Buch), pseudoleucaemia myelogenes (Runeberg), osteomyelitis maligna (Grawitz), ostosis sarcomatosa (Hammer), endothelioma intravascular (Markwald), lymphosarcoma multiplex ossium (Wieland), myelosarcoma (Schmaus), lymphadenia ossium (Nothnagel), erythroblastoma (Ribbert), plasmoma malignum (Hoffman).

case of chloroma; Vidal (58) from a case of tuberculous osteoarthritis; Kahler (54) from primary lympho-sarcoma of the spinal cord; Oerum (59) from a case whose bone tumors were multiple metastases of a gastric carcinoma. The case of osteomalacia which was reported by Jochman and Schumm (12) was subsequently shown to be one of multiple myeloma, and that of Askanazy (19), reported as one of lymphatic leukaemia, was undoubtedly one of multiple myeloma. However, von Jaksch (60) and also Fitz (61) have shown that proteosuria may be a marked feature of myxedema. Collins (62) reported a case of undoubted multiple myeloma that was observed for several months and in which there was no excretion of Bence Jones protein. Naunyn (63) reported a case in which the whole skeleton was riddled with metastatic carcinomatous growths. The urine of this case, although repeatedly examined for Bence Jones protein, was found to be free from that substance. Scheele and Herxheimer (64) reported a case of multiple myeloma with no Bence Jones protein in the urine.

The above mentioned case of Naunyn's may be explained, according to Weber, as follows: The tumor cells derived from bone marrow cells, however much they may resemble morphologically true bone marrow cells, are more prone to abnormality (including unusual degenerative changes) than real myelocytes. Furthermore, metastatic tumors in the bone marrow do not give rise to Bence Jones protein for the reason that non-myelogenic tumor cells are not affected in the same way.

The view of Decastello (5) that Bence Jones protein is excreted only by individuals with diseased kidneys is hard to reconcile with the statement of some that the serum proteins are *never*, and by others that they are *seldom* excreted with Bence Jones protein. One would think that if the kidneys are diseased, albuminuria would occur in a larger proportion of cases. Bence Jones protein is excreted in 80 per cent. of the cases of multiple myeloma, but it does not appear likely that so many would present kidney lesions. It seems more probable that the kidney lesions result from the excretion of Bence Jones protein rather than that they cause its elimination, especially since Stokvis has shown that hemiproteose after its subcutaneous injection passes through the kidneys without

doing them any apparent injury. If such injections are repeated frequently, however, the excreted hemiproteose excites organic disease in the kidneys.

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THE EFFECT OF THE CHEMICAL COMPOSITION OF THE MEDIUM ON THE LIFE CYCLE OF HYDATINA SENTA¹

A. FRANKLIN SHULL

(*Department of Zoölogy, Columbia University.*)

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I. INTRODUCTION

In several groups of animals there is a more or less regular alternation of parthenogenetic with sexual reproduction. A number of generations may be produced parthenogenetically, after which females appear that produce eggs requiring fertilization. Or parthenogenetic and sexual females may occur in varying proportions in the same generation. For one of these groups, the suborder Cladocera of the phyllopod Crustacea, commonly called "daphnians," the suggestion has several times been made that the chemical composition of the water in which the animals lived might affect the transition from one mode of reproduction to the other, but no very clear evidence has been adduced. In another group, the rotifers, in which a similar alternation of methods of reproduction exists, although no one has heretofore suggested that

¹ The major portion of the results here presented has been published in *The Journal of Experimental Zoology*, 1910, viii, pp. 311-354, and 1911, x, pp. 117-166.

chemical substances in the water play a rôle in determining the cycle, other external agents (temperature and nutrition) have been found inadequate to explain the observed phenomena. The rotifer most extensively used in the study of the influence of external agents on the mode of reproduction is *Hydatina senta*. Without taking into account facts not directly involved in the present problems, the life cycle of this rotifer may be stated as follows: From the resting (fertilized) egg there hatches invariably a female. This female may lay 40 to 50 eggs which are incapable of fertilization and always hatch into females. In this family, however, the females may be of two kinds. One kind, like their mother, produces only eggs that can not be fertilized and hatch into females; the other produces only eggs that can be fertilized, but if not fertilized, nevertheless develop and produce males. The females may therefore be spoken of as female-producers or male-producers. The former are parthenogenetic females; the latter may be called sexual females, for, while their eggs may develop parthenogenetically, they may on the other hand be fertilized. Both male-producers and female-producers may occur in the same family, but the proportion of each is subject to wide fluctuations. A family may consist entirely of female-producers, or (less commonly) only of male-producers, while every gradation between these extremes may be found. Maupas ('91) referred these great fluctuations to temperature differences, Nussbaum ('97) to nutrition; but Punnett ('06) and Whitney ('07) were unable to confirm either of these conclusions. It seemed highly desirable, therefore, to examine the possible influence of the chemical composition of the medium upon the life cycle. The following pages give the results of experiments which show that certain substances may exert a more or less marked influence upon the proportion of male-producers (sexual females).

II. DESCRIPTION OF THE EXPERIMENTS

I. Influence of substances introduced with the food on the percentage of male-producers. In the work of previous investigators, the results of which led to contradictory conclusions regarding temperature and nutrition, no account was taken of possible

chemical differences in the food cultures from which the rotifers were fed. The food used in my experiments consisted of various organisms reared in an infusion of fresh horse manure, about one

TABLE I.

Showing the number of male- and female-producers in the progeny of five sister individuals of Hydatina senta, one line being reared in spring water, the others in various concentrations of the filtrate from old food cultures. Male-producers are indicated by ♂♀, female-producers by ♀♀.

Spring Water		Old Culture Filtrate							
		One-fourth		One-half		Three-fourths		Undiluted	
♂ ♀	♀ ♀	♂ ♀	♀ ♀	♂ ♀	♀ ♀	♂ ♀	♀ ♀	♂ ♀	♀ ♀
12	14	5	37	6	39	4	29	0	46
2	9	3	34	0	22	1	16	0	24
2	11	0	38	0	44	0	29	0	19
0	19	6	34	0	31	0	41	0	20
0	32	1	17	9	31	1	41	0	15
1	31	0	47	0	5	0	36	0	7
2	0	4	40	0	32	0	35	0	30
5	13	1	27	0	42	0	18	0	28
1	20	5	24	0	31	0	16	0	35
1	28	0	42	0	18	2	36	0	19
		0	44	0	34	0	4	0	31
		0	23	0	21	0	27	0	38
						0	34	0	25
Total 26	177	25	407	15	350	8	362	0	337
Per cent. of ♂ ♀	12.8	5.7		4.1		2.1		0.0	

volume of manure to three volumes of water. It was conceivable that the manure solution itself might alter the cycle of the rotifers.

EXPERIMENT 9. The influence of substances found in the food cultures, as distinguished from the flagellate used as food, was tested as follows: An old manure culture, which had been made up with spring water, and which had been rejected about ten days before, was filtered through a Berkefeld filter. The filtrate was examined with a microscope and found to be free from protozoa. Rotifers were reared in various concentrations of this filtrate, one-fourth, one-half, three-fourths, and undiluted, as well as in pure spring water. The five lines were derived from sisters, and were fed equal quantities of food from the same fresh cultures. The

flagellate food lived readily in the filtrate, of all concentrations, and when the records were made two days later it was always abundant. Starvation, therefore, could play no rôle in the results. Table 1 shows the results.

A comparison of the totals shows that there was a gradual decrease, not only in the percentage of male-producers, but in their absolute number, from the line bred in pure spring water to that bred in the concentrated filtrate.

2. Influence of various undetermined constituents of feces on the percentage of male-producers. After it had been determined that a solution of horse manure could wholly prevent the appearance of male-producers, the immediate problem was to discover what constituent or constituents of the feces had this effect. Two methods of investigation were practicable. Substances which were known to be present in horse manure, or in feces in general, could be directly tested by experiment; or the solution of manure could be treated in such a way as to remove from it substances having given properties, after which the effect of the substances removed, or of those that remained, or of both, could be determined. By the latter method, the number of substances to be tried by the former method might be considerably reduced. It is this indirect method which is the subject of Experiments 19 to 22, inclusive. The manure solution was boiled; it was evaporated to dryness and the residue redissolved; it was evaporated to dryness and the residue extracted with ether and alcohol; and it was decolorized by boiling with animal charcoal. The following experiments show in detail the results of these operations.

EXPERIMENT 19. BOILING THE MANURE SOLUTION. After the manure solution from an old food culture, made up with spring water, had been filtered through a Berkefeld filter, part of the filtrate was boiled gently for four minutes. The loss of volume by evaporation was restored with distilled water. The remainder of the filtrate was not treated. The boiled filtrate was kept in stock and was boiled every day until exhausted, chiefly to prevent the development of bacteria. The unboiled filtrate was obtained daily just before using. Three pure lines of rotifers derived from sisters were reared in April, 1910, one in boiled filtrate, one in unboiled

filtrate, the third in spring water. The results are given in Table 2. The boiled filtrate has precisely the same effect as the unboiled. The substance in feces which prevents male-producers

TABLE 2.

Showing the number of male- and female-producers in the progeny of three sister individuals of Hydatina senta, one line being bred in boiled filtrate of old food cultures, one in unboiled filtrate, the third in spring water.

Spring Water			Unboiled Filtrate			Boiled Filtrate		
No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
1	27		0	7		0	42	
23	27		0	2		0	51	
5	46		0	4		0	47	
7	42		0	2		0	47	
1	34		0	57		0	32	
			0	26				
			0	42				
37	176	17.3	0	140	0.0	0	219	0.0

from appearing is not, therefore, an enzyme, nor bacteria, nor any other substance destroyed by boiling temperature.

EXPERIMENT 20. DRYING THE MANURE SOLUTION. A portion of the filtrate obtained from an old food culture, made of spring water as in the preceding experiment, was evaporated to dryness. The residue was redissolved in distilled water equal in volume to the original filtrate, and then boiled. One line of rotifers, bred from a female collected at Grantwood, N. J., in April, 1910, was reared in this redissolved filtrate. A second line, from a sister to the parent of the other line, was reared in the filtrate that had been simply boiled. Another line was bred in spring water. The data of the three lines are given in Table 3. The substance responsible for repressing the male-producers is not destroyed by drying and redissolving, for the redissolved filtrate has precisely the same effect as the merely boiled filtrate.

EXPERIMENT 21. ETHER- AND ALCOHOL-SOLUBLE PARTS OF MANURE SOLUTION. From an old food culture, made as in the preceding experiments from spring water, 125 c.c. were filtered through a Berkefeld filter, and the filtrate evaporated to dryness. The resi-

due was extracted with ether for twelve hours, after which the ether was filtered through paper and the solution evaporated to dryness. Less than 0.01 gram of ether-soluble substances was thus obtained. It did not seem likely that so small a quantity would have any noticeable effect on the proportion of male-producers,

TABLE 3.

Showing the number of male- and female-producers in the progeny of three sister individuals of Hydatina senta, one line being reared in the filtrate of old food cultures that had been dried and redissolved, one in boiled filtrate, and one in spring water.

Spring Water			Boiled Filtrate			Dried, Redissolved Filtrate		
No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
0	16		0	6		0	3	
3	11		0	15		0	42	
0	9		0	13		0	39	
15	20		0	12		0	43	
1	18		0	16				
			0	1				
			0	31				
19	74	20.4	0	94	0.0	0	127	0.0

but the experiment was nevertheless made. This ether-soluble residue was dissolved in 125 c.c. of distilled water, giving a colorless solution, and a line of rotifers was reared through four generations in it. The residue after ether-extraction was likewise dissolved in 125 c.c. of distilled water and boiled, making a brown solution not apparently different from the original manure culture, and a sister line of rotifers was reared in the solution. A third line was reared as control in spring water. Table 4 shows the result.

The experiment was so brief that the difference in the proportion of male-producers between the line in spring water and that in the ether-soluble part of the filtrate may mean nothing. The chance of obtaining any result from such a minute ether-soluble residue did not seem to warrant a more extensive experiment, especially since the part of the manure solution not soluble in ether had the same effect as the entire manure solution had in other experiments.

An experiment was started, in which the alcohol-soluble portion of the manure solution was obtained in a manner similar to the ether-extraction described. The portion soluble in absolute alcohol was smaller than that soluble in ether. The experiment was discontinued in the middle of the second generation, hence the detailed results are not of value. It may be said, however, that

TABLE 4.

Showing the number of male- and female-producers in the progeny of three sister individuals of Hydatina senta, one line being bred in a solution of the ether-soluble part of an old food culture, a second line in the part insoluble in ether, the third in spring water.

Spring Water			Ether Soluble Portion			Fraction Insoluble in Ether		
No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
0	16		0	29		0	4	
3	11		4	16		0	24	
0	9		5	44		0	20	
15	20		1	12		0	14	
1	18					0	43	
19	74	20.4	10	101	9.0	0	105	0.0

the first family reared in the alcohol-soluble portion contained a considerable number of male-producers.

EXPERIMENT 22. DECOLORIZED FILTRATE. It was noticed in the preceding experiments of this section that those residues which produced the same effect as the unaltered manure solution were brown like the original; those that had no effect were colorless. To determine whether the substance producing the brown color has any effect on the life cycle of the rotifers, a portion of the filtrate from old food culture was decolorized with animal charcoal. An excess of the charcoal was added to the filtrate, and the mixture boiled eight or ten minutes. It was then filtered through paper, and the volume of water lost by evaporation was restored, the added water being passed through the filter. More charcoal was added to the filtrate, and the boiling repeated. After three or four boilings the filtrate was practically colorless. It was not to be expected that the colored substance was the only one removed by this process for animal charcoal probably carries down most

substances to some extent. But if the colored substance is wholly responsible for the non-occurrence of male-producers in a manure solution, such a decolorized solution should yield the same proportion of male-producers as pure water. Whether it does or not may be seen from Table 5. The experiment was performed in May, 1910, with rotifers descended from a winter egg collected in Grantwood, N. J., in April, and kept in an ice-chest for a month.

Even the decolorized filtrate greatly reduces the proportion of male-producers, though not as much as the merely boiled filtrate

TABLE 5.

Showing the number of male- and female-producers in the progeny of three sister individuals of Hydatina senta, one line being reared in boiled filtrate of old food cultures, a second in filtrate decolorized with animal charcoal, the third in spring water.

Spring Water			Decolorized Filtrate			Boiled Filtrate		
No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
31	6		3	39		1	42	
20	5		3	29		0	9	
7	21		0	6		0	14	
6	23		8	27		0	22	
6	39		2	37		0	14	
16	14		1	30		0	38	
30	10		4	45		0	13	
0	41		0	48		0	20	
						0	5	
116	159	42.1	21	261	7.4	1	177	0.5

Whether the 7 per cent. difference between the decolorized filtrate and the boiled filtrate is due to total removal of the colored substance or to partial removal of some other substances, can not be decided from this experiment alone. That the latter is the case, at least in part, will appear later, when it is shown that certain other substances which are presumably present in the manure, and which were probably carried down mechanically by the charcoal, do reduce the percentage of male-producers. This does not show, however, that the colored substance may not also have the same effect to a slight extent.

The one male-producer in the first generation bred in the boiled filtrate is the only one I have ever obtained in undiluted filtrates

of old food cultures. This one individual need scarcely surprise us if we note the very high percentage of male-producers in the first two generations bred in spring water. Manure solution does not rigidly exclude male-producers. It is important to remember this in the consideration of other experiments where various substances do not wholly prevent male-producers from appearing.

3. Influence of alkalinity on the percentage of male-producers. EXPERIMENT 23. It was found impracticable to rear the rotifers in water that was even faintly acid, so several degrees of alkalinity were used. A $n/10$ solution of NaOH in distilled water was kept in stock in a tightly stoppered bottle. In making up this solution, no further precaution was taken to make the normality exact than to weigh the solid hydroxid carefully, exposed to the air. A $n/25$ solution of HCl was made up by titration against the NaOH solution immediately after the latter was prepared, and was kept in stock. Three grades of alkalinity were used in the experiment, the solutions being prepared as follows: Great Bear spring water, which is itself alkaline, was used unaltered in one line; for the second line, the $n/10$ NaOH solution was diluted to ten times its volume with Great Bear water; for the third line, the $n/25$ HCl solution was diluted to forty times its volume with Great Bear water. The alkalinity of the Great Bear water was not accurately determined, but was less than $n/10$, so that addition of $n/10$ NaOH solution increased its alkalinity; neither was the alkalinity of the diluted solutions known. As it was necessary to expose the NaOH solution to the air while it was being used, the dilute solution was made up daily. The data from this experiment are given in Table 6, where the alkalinity increases from left to right.

From this experiment it would seem that the greater the alkalinity the fewer the male-producers, but the differences are too small to draw a safe conclusion from one experiment.

EXPERIMENT 24. The stock solutions in this experiment were produced in essentially the same manner as in the preceding experiment, but in diluting them for daily use distilled water was used instead of Great Bear water. The alkalinity of the final solutions was therefore more accurately known. It could not be exactly

known, however, for the food cultures were of varying alkalinity; and, because the protozoa in them were not always equally abundant, a variable quantity of the cultures was necessarily used. Before adding food to the dishes, the three solutions used were respectively $n/200$ NaOH, neutral (distilled water), and $n/300$ HCl. After adding food, the acid solution was practically neutral or faintly alkaline, while the alkalinity of the other two solutions

TABLE 6.

Showing the number of male- and female-producers in the progeny of three sister individuals of Hydatina senta, one line being bred in Great Bear spring water, a second in water less alkaline, and the third in water more alkaline, than Great Bear water.

Lowest Alkalinity			Great Bear Water			Highest Alkalinity		
No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
0	31		7	24		1	36	
0	27		0	21		3	31	
14	16		0	18		0	39	
0	16		0	18		0	3	
5	18		2	22		1	5	
0	26		11	4		11	15	
0	3		0	32		0	24	
0	10		0	40		0	10	
0	27		7	40		1	21	
6	27		1	25		0	2	
1	9							
26	210	11.0	28	244	10.2	17	186	8.3

was slightly altered. In Table 7 the columns are designated according to the acidity or alkalinity of the solutions before adding food but it should be remembered that an actually acid solution was never used.

While the highest percentage of male-producers is found as before in the lowest degree of alkalinity, there is not the regular decrease in the proportion of male-producers with increase of alkalinity, such as was seen in the preceding experiment. As it would not be practicable to rear the rotifers in much more alkaline water, all that we may safely conclude is that if alkalinity influences the proportion of male-producers it does so to only a small extent.

TABLE 7.

Showing the number of male- and female-producers in the progeny of three sister individuals of *Hydatina senta*, one line being reared in $n/200$ NaOH, a second in distilled water and one in $n/300$ HCl (but see text).

$\frac{n}{300}$ HCl			Distilled Water			$\frac{n}{200}$ NaOH		
No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
1	35		0	47		3	28	
0	23		1	32		3	52	
1	10		5	47		6	46	
13	38		3	42		4	48	
5	20		1	22		0	48	
0	15		0	55		0	49	
1	21		4	34		1	25	
0	32		0	48		4	34	
			5	40				
21	194	9.7	19	367	4.9	21	330	5.9

4. Influence of urea on the percentage of male-producers.

EXPERIMENT 25. One line of rotifers was bred in a $m/400$ solution of urea, a control line derived from a sister individual was reared in spring water. The experiment was performed twice, A and B, in Table 8. In A, the parents of both lines had been in spring water before the beginning of the experiment; in B, both had been in urea. This experiment seems to indicate that urea in the water tends to reduce the proportion of male-producers.

5. Influence of ammonium compounds on the percentage of male-producers. EXPERIMENT 26. AMMONIUM SALTS. Four sister individuals became the parents of the four lines in this experiment, which was performed in June, 1910. One line was reared in spring water, the others in ammonium salts of the following strengths: $m/500$ NH_4Cl , $m/500$ NH_4NO_3 , and ammonium carbonate 1 gram to 7500 c.c. of the solution. The carbonate was not computed in terms of molecular solution, because the c.p. salt was not used. The substance taken was probably what is known as the sesquicarbonate, a mixture of the bicarbonate and the carbamate. Table 9 gives the details of each line.

All of these ammonium salts reduced the proportion of male-producers, two of them to one-half the proportion obtained in spring water, the other to one-third. The consistent results from

TABLE 8.

Showing the number of male- and female-producers in the progeny of two sister individuals of *Hydatina senta*, one line being bred in spring water, the other in a solution of urea. A and B are separate experiments.

Experiment	Spring Water				$\frac{m}{400}$ Urea			
	No. of Generation	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
A.....	1	0	2		1	0	4	
	2	0	44		2	1	28	
	3	0	3		3	0	5	
	4	0	34			0	4	
	5	5	32			0	10	
	6	7	24		4	2	34	
	7	1	39		5	0	40	
	8	0	3		6	0	33	
		3	35		7	1	46	
	9	1	7		8	0	4	
		0	2			0	2	
	10	0	7			7	24	
		0	17		9	0	1	
	11	1	47			0	1	
	12	11	42		10	0	12	
	13	11	26		11	1	16	
	14	9	43		12	5	51	
	15	6	39		13	1	33	
	16	6	37		14	2	42	
	17	4	44		15	0	1	
	18	1	43			0	2	
Total		66	570	10.3		20	473	4.0
B.....	1	5	11		1	5	51	
	2	1	49		2	1	33	
	3	17	35		3	2	42	
	4	0	23		4	0	1	
	5	5	25			0	2	
	6	5	6			0	18	
Total		33	149	18.1		8	209	3.6

the three salts seemed to make a repetition of the experiment with any one of them unnecessary.

EXPERIMENT 27. AMMONIUM HYDROXID. On July 6, 1910, a female from the same pure line as those of the preceding experiment was placed in a solution of ammonium hydroxid, made by diluting strong NH_4OH to 5000 times its volume with Great Bear spring water. Its progeny for thirteen generations were bred in

TABLE 9.

Showing the number of male- and female-producers in the progeny of four sister individuals of *Hydatina senta*, one line being bred in spring water, the other three respectively in solutions of ammonium chloride, ammonium carbonate, and ammonium nitrate.

Spring Water		Ammonium Salts					
		Chlorid		Carbonate		Nitrate	
No. of ♂ ♀	No. of ♀ ♀	No. of ♂ ♀	No. of ♀ ♀	No. of ♂ ♀	No. of ♀ ♀	No. of ♂ ♀	No. of ♀ ♀
30	19*	1	15	4	23	10	28*
2	17*	8	26	7	8*	11	1*
0	2*	7	4*	2	11		
8	17	3	25	1	0	4	20
6	22	11	14		0	6	25
22	29	3	29	22	6	2	39
35	19	0	38	0	6	8	25
22	12	1	8	0	13	8	35
11	17	1	38	10	17	1	13
25	19	3	34	2	4	3	42
19	26	8	44	4	35	11	22
20	35	0	33	0	35	0	7
10	25	0	18	0	35	12	15
15	21	1	18	6	32	0	16
7	16	13	16	0	19		
				12	31		
				10	15		
232	296	60	360	80	290	76	288
Per cent of ♂ ♀43.9		14.2		21.6		20.8	

the same solution. The strength of the original hydroxid was not known. Table 10 shows this line, and a control in Great Bear spring water.

The ammonium hydroxid, like the salts, reduced the proportion of male-producers to less than half that of the line in spring water. Here again it seemed unnecessary to repeat the experiment.

6. Influence of beef extract on the percentage of male-producers. The experiments with beef extract were designed chiefly to test the question whether extractives which are present in feces, influence the proportion of male-producers. Liebig's extract was used, because of the absence from it of certain classes of substances which are present in other brands.

EXPERIMENT 28. Two sister females were isolated June 23,

*Remainder of family not recorded.

TABLE 10.

Showing the number of male- and female-producers in the progeny of two sister individuals of Hydatina senta, one line being bred in dilute ammonium hydroxid solution, the other in spring water.

Spring Water				Ammonium Hydroxid			
No. of Generation	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
1.....	4	27		1	0	34	
2.....	8	23		2	0	25	
3.....	0	29		3	0	7	
4.....	0	12		4	0	27	
5.....	2	11		5	7	28	
6.....	4	28		6	12	14	
7.....	14	21		7	0	24	
8.....	15	21		8	4	22	
9.....	14			9	1	20	
	2	30		10	1	18	
	9	31		11	0	8	
10.....	3	40		12	6	34	
	4	15			0	15	
11.....	16	19		13	3	50	
	0	24					
12.....	4	24					
13.....	0	18					
14.....	22	11					
Total.....	121	409	22.8		34	326	9.4

1910, from one of the groups in other experiments in progress at that time. One was placed in a solution of Liebig's beef extract, the other in spring water. The beef extract was dissolved in spring water, and a 1 per cent. solution kept in stock. This stock solution was boiled once or twice a day to prevent the growth of bacteria, and in the intervals was set in the cool water of a spring. Loss by evaporation was restored with distilled water. The stock solution was diluted with Great Bear spring water when ready to be used. In the first experiment, Table 11, A, a 0.03 per cent. solution was used for the first two generations, a 0.04 per cent. solution thereafter. In B, a female was removed from the line in beef extract in A to spring water, so that here also the strength of the beef extract is 0.04 per cent., the line in the extract being the last part of the corresponding line in A.

In both experiments the number of male-producers was much smaller in the beef extract than in the spring water.

TABLE II.

Showing the number of male- and female-producers in the progeny of two sister individuals of *Hydatina senta*, one line being bred in spring water, the other in a solution of beef extract. A and B are separate experiments.

Experiment	Spring Water				Beef Extract			
	No. of Generation	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀	Per Cent. of ♂ ♀
A.....	1	7	16		1	2	25	
	2	14	17			1	19	
	3	17	41		2	3	22	
	4	8	45			5	39	
	5	1	45		3	0	31	
	6	31	29			1	27	
	7	9	13		4	1	33	
	8	8	31			0	5	
	9	17	30		5	1	29	
	10	4	27			0	31	
	11	8	23		6	0	20	
	12	0	29			0	14	
	13	0	12		7	2	40	
Total.....		124	358	25.7		27	630	4.1
B.....	1	0	37		1	6	36	
	2	10	19			0	54	
	3	1	17		2	0	23	
		0	20			0	18	
	4	0	19		3	0	20	
		7	27			0	25	
Total.....		18	139	11.4		7	226	3.0

EXPERIMENT 29. The preceding experiment was repeated, but this time two strengths of the extract were used, one a 0.04 per cent., the other a 0.05 per cent. solution. Both lines are represented in Table 12. The results of these repetitions agree closely with those of the first experiment, showing even a smaller percentage of male-producers in the stronger solution of the extract. There can scarcely be any doubt that beef extract tends to reduce the proportion of

TABLE 12.

Showing the number of male- and female-producers in the progeny of three sister individuals of *Hydatina senta*, one line being bred in spring water, the other two in solutions of beef extract of different strengths.

Spring Water			0.04 Per Cent. Beef Extract			0.05 Per Cent. Beef Extract		
No. of Generation	No. of ♂ ♀	No. of ♀ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀
1.....	8	23	1	0	21	1	0	20
2.....	0	29	2	0	23	2	0	13
3.....	0	12	3	0	15	3	0	9
4.....	2	11		0	17		0	4
5.....	4	28	4	0	13	4	0	11
6.....	14	21		0	15		1	12
7.....	15	21	5	0	26	5	0	18
8.....	14	25	6	0	19		0	19
	2	30	7	1	34	6	0	34
	9	31	8	4	34		0	13
9.....	3	40	9	1	38	7	0	30
10.....	16	19	10	0	20		0	37
						8	0	35
							0	24
						9	1	14
							0	33
Total.....	87	290		6	275		2	326
Per cent. of ♂ ♀	23.0			2.1			0.6	

male-producers, and it seems quite possible that these could even be wholly excluded by sufficiently strong solutions of the extract.

7. Influence of creatin on the percentage of male-producers.

The positive results from the beef extract in the experiments of the preceding section suggested, among other things, that extractives may alter the proportion of male-producers. One of the commoner extractives, creatin, was selected to put this matter to the test. The creatin used in these experiments was not pure, but contained an admixture of creatinin.

EXPERIMENT 30. From three sister females, three lines were reared, starting on July 18, 1910. One line was reared in a 0.02 per cent. solution of the crude creatin in Great Bear spring water, one in a 0.025 per cent. solution, the third in spring water alone. Table 13 shows the three pure lines in detail.

EXPERIMENT 31. This is a repetition of Experiment 30, but with weaker solutions of creatin in both parts, a 0.01 per cent. and a 0.015 per cent. solution being used. In both of these experiments

some of the eggs laid in the creatin solution did not hatch in the usual short time required in spring water. In such cases the creatin

TABLE 13.

Showing the number of male- and female-producers in the progeny of three sister individuals of Hydatina senta, one line being bred in spring water, the other two in creatin solutions of different strengths.

Spring Water			0.02 Per Cent. Creatin			0.025 Per Cent. Creatin		
No. of Generation	No. of ♂ ♀	No. of ♀ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀
1.....	14	25	1	0	7	1	0	24
	2	30	2	0	11	2	0	28
	9	31	3	0	16		0	27
2.....	3	40	4	8	27	3	0	22
	4	15		0	3	4	0	17
3.....	16	19	5	0	29	5	0	16
	0	24		0	17	6	0	17
4.....	4	24	6	1	24		1	33
5.....	0	18		0	38	7	1	24
6.....	22	11	7	0	39	8	0	36
7.....	4	36		0	27	9	1	36
8.....	9	31	8	0	32	10	0	16
9.....	1	21		0	27	11	0	27
10.....	6	36	9	0	25		0	15*
11.....	7	14*		0	20	12	1	16*
			10	1	28*			
				0	18			
			11	0	17*			
Total.....	101	375		10	405		4	354
Per cent. of ♂ ♀.....	21.2			2.4			1.1	

was diluted after the death of the parent, and many of the eggs then hatched. Table 14 presents the details of the experiments.

In both of these tables the same result appears, that is, a lower percentage of male-producers in the creatin solution than in spring water, and a lower percentage in the stronger solution than in the weaker. The differences are as great as were those of the beef extract, and are too marked to leave any doubt regarding the influence of creatin.

III. SUMMARY AND CONCLUSIONS

From the results of the foregoing experiments it seems clear that certain chemical compounds in the water may greatly modify the

*Remainder of family not recorded.

TABLE 14.

Showing the number of male- and female-producers in the progeny of three sister individuals of *Hydatina senta*, one line being reared in spring water, the other two in creatin solutions of different strengths.

Spring Water			0.01 Per Cent. Creatin			0.015 Per Cent. Creatin		
No. of Generation	No. of ♂ ♀	No. of ♀ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀	No. of Generation	No. of ♂ ♀	No. of ♀ ♀
1.....	4	24	1	1	13	1	2	14
2.....	0	18		0	25	2	0	28
3.....	22	11	2	2	46		0	18
4.....	4	36	3	3	39	3	0	19
5.....	9	31	4	0	43		0	18
6.....	1	21	5	0	25	4	0	28
7.....	6	36	6	0	22	5	0	36
8.....	7	14*		0	15	6	0	30
9.....	0	7*	7	0	30*	7	0	16*
				5	28*			
Total.....	53	198		11	286		2	207
Per cent. of ♂ ♀.....	21.1			3.7			0.9	

proportion of male-producers in *Hydatina*. The conclusions reached may be summarized as follows:

An extract of horse-manure may wholly prevent male-producers from appearing. Boiling such a manure extract does not diminish its effect, neither does drying and redissolving it.

The substance in manure extract which prevents male-producers from appearing is apparently insoluble in ether and absolute alcohol.

The brown colored substance of manure extracts probably does not affect the proportion of male-producers.

The degree of alkalinity, when comparatively small, seems to have a determining influence; but the differences obtained were slight and the results were not uniform.

Urea tends to reduce the proportion of male-producers. Other substances having the same effect are ammonium hydroxid and three ammonium salts: the chlorid, the carbonate, and the nitrate.

Beef extract and creatin solutions greatly reduce the proportion of male-producers.

*Remainder of family not recorded.

From the purely biological standpoint, considerable interest attaches to these results, because of the possible explanation they give of the conflicting results of earlier workers. At least some of the chemical substances tested in the experiments are to be found in the food-cultures from which the animals are fed. If an attempt is made to starve the rotifers, a smaller quantity of the chemical substances, as well as a smaller quantity of food, is administered. Since the effect of these substances is to reduce the proportion of male-producers, the supposed starvation (diminution in the amount of certain substances) should increase that proportion. Experiment shows that it does. It is not improbable, therefore, that effects attributed to diminished nutrition may really be due to decreased quantities of chemical substances.

From the standpoint of both chemistry and biology, a very important question remains unanswered. It is not known how the substances effect the reduction of the proportion of male-producers. In general, two methods are conceivable. It seems now almost certain, from other experiments, that once an egg is laid the nature of the female hatching from it can not be changed by subjecting the egg or the resulting individual to manure solution. The chemical substances in solution, therefore, may alter certain fundamental processes in a given egg or oogonium, during growth and maturation or at some other period, so that the individual resulting from that egg becomes a female-producer, whereas in the absence of those substances the same egg might have given rise to a male-producer. On the other hand, the eggs or oogonia may be differentiated into two classes by a process in nowise dependent upon the chemical nature of the medium; if this be true, the substances that affect the cycle may be more toxic to one class of eggs than to the other, and prevent many of the former class from being laid. Experiments are now in progress which bid fair to determine which of these hypotheses is correct, but the conclusions to be drawn from them can not be predicted.

Valuable suggestions were received during the progress of this work from Prof. William J. Gies and Prof. T. H. Morgan.

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A STUDY OF LINTNER SOLUBLE STARCH¹

ERNEST D. CLARK

(Laboratory of Biological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.)

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I. INTRODUCTION

About three years ago Meyer² used Lintner soluble starch as the substrate in some experiments on the quantitative determination of diastatic activity. The degree of hydrolysis in Meyer's earlier estimations was ascertained from the extent to which Fehling solution was reduced under certain uniform analytic conditions. Sub-

¹ A preliminary report of this investigation was published in the *Journal of Biological Chemistry*, 1909-10, vii, *Proceedings of the American Society of Biological Chemists*, p. lv.

² Meyer: Determination of diastatic power, etc. Dissertation, Columbia University, 1908.

sequently, Meyer discovered that his preparation of Lintner soluble starch possessed striking reducing power. He concluded from this fact that soluble starch is a reducing substance. Accordingly, he subtracted from the weight of the cuprous oxid obtained in his later diastatic determinations the weights of the cuprous oxid that the employed amounts of Lintner starch were initially able to precipitate from Fehling solution under the conditions of the uniform reduction tests.³

Prior to the publication of Meyer's results, we were under the impression that true soluble starch is devoid of reducing power. Lintner's method for the preparation of soluble starch appeared to us to yield a crude product. Meyer's preparations of Lintner soluble starch seemed to be mixtures of substances. We undertook this work for the purpose of ascertaining the facts in these and related connections.

We prepared Lintner soluble starch in accordance with the directions published by Meyer,⁴ which, although detailed, are not always clear. On this account our procedure may have differed here and there from Meyer's, but we are confident that any such unavoidable variations were too slight to influence the results to any material extent.

II. EXPERIMENTAL

1. **Origin of the potato starch employed in these experiments.** Our preparations of soluble starch were made from commercial potato starch and also from starch obtained by us from potatoes.⁵ The latter preparations were made by macerating potatoes on a grater, thoroughly mixing the pulp with an excess of water, then straining the milky liquid through muslin and finally allowing the starch to settle out. The starch sediment was freed from the supernatant extract by decantation, then washed with about

³ It is evident that such a subtraction was warranted only if the reduction was due to an impurity that neither gained nor lost reducing power as hydrolysis progressed. Meyer, however, attributed the reducing power to the *substrate itself*, and not to an impurity.

⁴ Meyer: loc. cit.

⁵ By making our own starch we sought to eliminate the influence of any impurities that might be introduced into commercial starch by processes of manufacture.

five liters of distilled water on a Büchner funnel and allowed to dry at 20° C. Two samples of our own potato starch were prepared in this way. We also washed and dried, in the same manner, two specimens of commercial potato starch.

2. Preparation of Lintner soluble starch. We prepared Lintner soluble starch from each of the four available specimens of potato starch. Eighty five grams of each product were placed in liter bottles and allowed to stand in 750 c.c. of 7.5 per cent. hydrochloric acid solution for seven and one-half days, with occasional shaking. Each product was then washed with 25 liters of distilled water, but despite this treatment the moist starch granules gave an acid reaction when placed on sensitive litmus paper. The samples were then dried for twenty hours in air which was rendered free from dust and fumes. This product dissolved in water at 90° C. with little or no gelatinization. Under the microscope, the product appeared to have the structure of untreated starch. The properties of each product are discussed below.

PREPARATION 1. FROM COMMERCIAL STARCH (A). We washed the material with 25 liters of distilled water. We suspended 5 grams of dry commercial starch (A), after the above treatment with acid, in 100 c.c. of cold water and titrated with $n/100$ sodium hydroxide solution using rosolic acid as the indicator. The starch showed an acidity equal to the alkalinity of 14 c.c. of $n/100$ sodium hydroxide solution. When samples were suspended in 100 c.c. of water and heated to 40°, 50° and 70°, and then filtered, the filtrates became bright red, purple, and deep blue, respectively, when iodine solution was added to them. No further attempt was made to wash out the acid. This specimen of soluble starch was subsequently used to show the effects of acid in the preparation of *solutions* of Lintner soluble starch.

PREPARATION 2. FROM COMMERCIAL STARCH (B). This specimen of starch (B) was washed with 75 liters of distilled water on a Büchner funnel and was allowed to stand over night in one liter of water treated with 1 c.c. of ammonium hydroxide solution of 0.90 sp. gr. The product was washed the next day with 25 liters of distilled water and dried as in the case of Preparation 1. Five

grams of this dried specimen in 100 c.c. of water required 1.6 c.c. of $n/100$ sodium hydroxide solution for neutralization. This preparation was then allowed to stand over night with one liter of water containing 1 c.c. of ammonium hydroxide solution of 0.96 sp. gr. It was next washed with 25 liters of distilled water on a Büchner funnel. After its desiccation in the usual manner, 5 grams of the dry material in 100 c.c. of water required only 0.65 c.c. of $n/100$ sodium hydroxide solution for neutralization. The acidity of this product was as low as that of Meyer's best preparations.

PREPARATION 3. FROM OUR OWN STARCH (*A*). We washed this specimen (*A*) of our own starch with 60 liters of distilled water. Washing with the last 30 liters was by decantation. Five grams of this dry specimen in 100 c.c. of water required 9 c.c. of $n/100$ sodium hydroxide solution for neutralization. The main bulk of the material was then washed by decantation with 30 liters more of distilled water. Five grams of the material in 100 c.c. of water now required 6.6 c.c. of $n/100$ sodium hydroxide solution for neutralization. The product was allowed to stand for four hours in one liter of distilled water treated with 1 c.c. of ammonium hydroxide solution of 0.96 sp. gr., and then was washed with 20 liters of water on a Büchner funnel. Five grams of the material in 100 c.c. of water required 0.9 c.c. of $n/100$ sodium hydroxide solution for neutralization.

PREPARATION 4. FROM OUR OWN STARCH (*B*). This specimen (*B*) of our own starch was treated exactly like Preparation 3 and had a final acidity equal to the alkalinity of 0.8 c.c. of $n/100$ sodium hydroxide solution.

Preparations 2-4, inclusive, after treatment with water at 40°, 50° and 70°, gave the colors with iodine that were produced after similar treatment of Preparation 1.

3. **Dialysis experiments with soluble starch solutions.** EXPERIMENTS WITH SOLUTIONS IN COLD WATER. All the preparations of soluble starch were subjected to dialysis. Two gram portions were suspended in 50 c.c. of cold distilled water, the suspensions were transferred to collodion bags each of which was immersed in

50 c.c. of distilled water. In every case these bags were very carefully tested for defects, and only perfect ones were used.⁶

The dialysis was allowed to proceed for sixteen hours in each case, when portions of each of the four diffusates were subjected to tests by the reagents with the results indicated below:

	<i>Carbohydrate.</i> Molisch test	<i>Polysaccharid.</i> Iodine solution	<i>Reducing substance.</i> Fehling solution	<i>Chlorin.</i> Silver nitrate solution
Diffusate from Preparation 1....	Strong	Bright red	Reduction	None
Diffusate from Preparation 2....	Strong	Bright red	No reduction	None
Diffusate from Preparation 3....	Strong	Bright red	Reduction (?)	None
Diffusate from Preparation 4....	Weaker	Bright red	No reduction	None

Two gram portions of the soluble starch preparations were then suspended separately in 50 c.c. of cold water and the mixtures allowed to stand for periods of time equal to those of the dialysis treatment in the foregoing experiments. The liquid in each sus-

⁶ Such bags may be readily prepared by the following method now in general use in this laboratory: Pour into a *dry*, clean, Erlenmeyer flask (250 c.c. capacity) about 25 c.c. of fairly thick solution of collodion in alcohol and ether. Gradually pour the solution back into the bottle containing the collodion supply, but *slowly turn* the inclined, *inverted* flask on its longitudinal axis (while the liquid runs out), in order to effect complete covering of the inner surface of the flask with a layer of the solution. Keep the inverted flask inclined over the mouth of the bottle of collodion solution and *continuously* revolve the flask *slowly* on its longitudinal axis *so long as collodion drips from the rim of the flask*. Then surround the *upright* flask with the hands, in order to warm it, and thus (by favoring evaporation of the solvent) hasten the solidification of the thin, tough, collodion lining.

So soon as the collodion skin on the rim of the flask is dry and stiff (about fifteen minutes after the conclusion of the foregoing process), cut or carefully scrape it off, or loosen it with a finger nail, at that part of the flask, so as to make a free edge at the mouth of the contained collodion bag. Cautiously slip the tip of a knife blade behind the thin delicate membrane in the flask, and allow about 25 c.c. of water to run down between the bag and the flask. Shake the *inclined* flask gently *while it is turned on its longitudinal axis*. The membrane can readily be detached from the flask in this manner. If necessary, press the rounded end of a stirring rod against the exterior surface of the bag above any point of special adhesion to the glass. Finally withdraw the detached bag cautiously. In pulling on the bag, avoid complete closure of its mouth. Fill the removed bag with water, wipe its exterior dry with filter paper and determine definitely whether or not the bag leaks. The bag may be translucent and defective if the collodion solvent is not completely eliminated before the treatment with water is begun. (This description of the method of preparing collodion bags is taken from Dr. Gies' multigraphed directions for laboratory work in physiological chemistry.)

pension was then filtered through a hardened filter and the clear filtrate subjected in each case to the tests previously applied to the dialysates. The results were the same. In every case, all the tests were made, in blank, on distilled water in which we had previously immersed the collodion bags or the filter papers employed in the various treatments. In this way we guarded against erroneous conclusions due to accidental contamination of our solutions with substances from the paper or collodion. Blank Fehling tests were made in every case in which that reagent was used.

The foregoing experiments were repeated in every detail two months later and the following results obtained:

	<i>Polysaccharid.</i> Iodine solution	<i>Reducing substance.</i> Fehling solution
Diffusate from Preparation 1.....	Bright red	Reduction
Diffusate from Preparation 2.....	Bright red	Reduction
Diffusate from Preparation 3.....	Bright red	Reduction
Diffusate from Preparation 4.....	Bright red	Reduction

EXPERIMENTS WITH SOLUTIONS IN HOT WATER. All the results just described were obtained by the use of suspensions of soluble starch in *cold* water. In the Lintner diastatic method the soluble starch is always dissolved, with the aid of heat, in water rendered neutral to rosolic acid. Therefore, we repeated the dialysis experiments with soluble starch under the conditions that prevail in the Lintner diastatic determinations. First, we titrated 100 c.c. portions of our distilled water with $n/100$ sodium hydroxide solution, using rosolic acid as the indicator. The larger volumes of the same supply of distilled water were treated with sufficient $n/100$ sodium hydroxide solution to exactly neutralize the water. Two gram portions of each preparation of soluble starch were suspended in 25 c.c. of cold neutral water and poured into 75 c.c. of neutral water at 95° C. The resultant mixture was kept at 95° for 30 minutes, and then was made up to exactly 100 c.c. These solutions were somewhat opalescent but clear. One-half of each of these volumes of soluble starch solution was dialyzed as before, with 50 c.c. of neutral water on the outside of the membrane in each case. The usual blank tests were also made, with negative results, for all the filter paper and collodion used. Dialysis was continued for twelve

hours in each experiment. Tests applied to the water surrounding the bags then gave the following results:

	Polysaccharid, Iodine solution	Reducing substance, Fehling solution
Diffusate from Preparation 1.....	Purple, changing to blue with excess of iodine	Reduction
Diffusate from Preparation 2.....	Purple, changing to blue with excess of iodine	No reduction
Diffusate from Preparation 3.....	Purple, changing to blue with excess of iodine	No reduction
Diffusate from Preparation 4.....	Purple, changing to blue with excess of iodine	No reduction

4. Fractional precipitations with magnesium sulfate. The results described above, as well as those which were obtained on the dialysates and which are outlined below, made it evident that our solutions of Lintner soluble starch contained several carbohydrates. We concluded to attempt fractional separation of these substances and successfully employed magnesium sulfate for that purpose.

DIFFUSATES. The dialysates from Lintner soluble starch solutions which had been obtained in *cold* water in the manner described above did not yield precipitates when the diffusates were saturated with magnesium sulfate, although the diffusates gave red colorations when treated with iodine solution. Similar results were obtained with diffusates from such starch solutions in dialysis experiments of longer duration (24 hours) with 10 c.c. of external water instead of 50 c.c. as in the previous experiments.

We then tried the effect of magnesium sulfate on the more concentrated diffusates obtained in the dialysis of aqueous solutions of Lintner soluble starch prepared with the aid of heat. A series of dialysis experiments was made in the manner already indicated upon starch solutions prepared at temperatures of 95° (as before) and 75°. The latter temperature was found to be just sufficient to effect solution of the soluble starch and also to produce a water-clear fluid.

After a dialysis period of twenty hours, each of the diffusates from the soluble starch solutions made at both 75° and 95° showed the following properties: deep blue color with iodine solution, precipitate upon the addition of an excess of alcohol, and reducing action upon Fehling-Benedict solution.

Each of the dialysates from the soluble starch solutions prepared at both temperatures was then saturated with magnesium sulfate. A *precipitate* was produced in each instance. It was filtered off, washed with a saturated solution of magnesium sulphate, redissolved in pure water and treated with dry sodium carbonate in quantities just sufficient to precipitate the magnesium as carbonate. This precipitate was removed by filtration. The filtrate yielded a *deep blue* color with iodine solution and slightly reduced Fehling-Benedict solution.

The *filtrates* from the carbohydrate precipitated by magnesium sulfate were treated with sodium carbonate for the precipitation of the magnesium as carbonate. After removal of the latter by filtration, each filtrate yielded a *red* color with iodine solution and reduced Fehling-Benedict solution. Saturation of these filtrates with ammonium sulphate failed, in each case, to produce further precipitation.

These experiments with typical diffusates involving fractional precipitation with magnesium sulfate were repeated several times and all the results confirmed. This process has shown that Lintner soluble starch, when dissolved in water and subjected to dialysis, yields to the diffusate a mixture of substances including one or more erythrodestrins.

DIFFUSION RESIDUES. The solutions remaining in the bags at the conclusion of dialysis were tested in each instance with iodine solution and also with Fehling solution. The former reagent produced the same colorations, respectively, as those indicated on page 200, while the Fehling solution was reduced in each case.

5. Fractional precipitation with alcohol. **ALCOHOLIC PRECIPITATES FROM DIFFUSION RESIDUES.** It was obviously desirable to subject the solutions in the bags to further investigation. Each of these solutions was poured into 400 c.c. of 95 per cent. alcohol and, after thoroughly shaking them, the mixtures were allowed to stand undisturbed for a month. Each fluid was, from the beginning, an opalescent colloidal solution which did not show any tendency toward agglutination even during this long period. It was found, however, that a *single drop* of 10 per cent. sodium chloride solution sufficed, in each case, to induce instantaneous precipita-

tion of soluble starch in large flocky masses.⁷ The heavy precipitate was filtered off and both it and the clear filtrate were saved for the tests mentioned below.

The isolated precipitates of soluble starch, when immersed in 50 c.c. of *cold* water, dissolved completely in a few minutes. Each such solution was then poured into 400 c.c. of 95 per cent. alcohol, and the mixture allowed to stand for a week. Reprecipitation with a drop of saline solution was then effected and the soluble starch precipitate in each case, after being filtered off, was spread on a glass plate and dried before an electric fan. Each desiccated product was a very friable amorphous mass.

One-half of a gram of the dry product in each case was suspended in 10 c.c. of cold water and allowed to stand over night. The next morning a small quantity of undissolved matter was filtered off and the clear filtrate tested by the reagents and with the results shown below:

	<i>Polysaccharid.</i> Iodine solution	<i>Reducing substance</i> ⁸ . Fehling solution
Reprecipitated Product 1.....	Purple	Reduction
Reprecipitated Product 2.....	Purple	Reduction
Reprecipitated Product 3.....	Blue	Reduction
Reprecipitated Product 4.....	Blue	Reduction

ALCOHOLIC FILTRATES FROM DIFFUSION RESIDUES. *First series.* The alcoholic filtrates from the precipitates of soluble starch in the cases of products 3 and 4 were now evaporated to dryness on water-baths. Each residue was dissolved in a small volume of water and the solutions tested by the methods and with the results indicated in the following summary:

	<i>Polysaccharid.</i> Iodine solution	<i>Reducing substance.</i> Fehling solution
Filtrate from reprecipitated product 3.....	Red	Reduction
Filtrate from reprecipitated product 4.....	Red	Reduction

The soluble starch solutions that remained in the bags at the conclusion of a *second* series of dialysis experiments were given the treatment with alcohol, saline solution, etc., which has just been

⁷ Dr. Gies has been obtaining similar results with various colloids in alcoholic solution, protein salts among them.

⁸ Blank tests were made as usual and also one on the alcohol used, to see if any reducing substances were present. The blank tests were negative in every case.

described. One-half gram portions of the dry substance were dissolved in 10 c.c. volumes of distilled water. The fluids were tested by the reagents and with the results indicated in the appended summary:⁹

	<i>Polysaccharid.</i> Iodine solution	<i>Reducing Substance.</i> Fehling solution
Reprecipitated product 1.....	Purple	Reduction
Reprecipitated product 2.....	Blue	Reduction
Reprecipitated product 3.....	Blue	Reduction
Reprecipitated product 4.....	Blue	Reduction

Second series. In the first series of experiments with the alcoholic filtrates from the soluble starch precipitates the alcohol was removed by evaporation at ordinary steam-bath temperature. Fearing that this temperature had caused some degree of hydrolysis, we sought, in the second series of tests, to prevent such a change by evaporating the alcoholic filtrates at 40°. We took up each residue in 10 c.c. of water at 40° and tested the solution by the methods and with the results indicated below:

	<i>Polysaccharid.</i> Iodine solution	<i>Reducing substance.</i> Fehling solution
Filtrate from reprecipitated product 1...	Reddish brown	Very strong reduction
Filtrate from reprecipitated product 2...	Yellow brown	Very strong reduction
Filtrate from reprecipitated product 3...	Reddish brown	Strong reduction
Filtrate from reprecipitated product 4...	Reddish brown	Strong reduction

Soluble starch preparations 3 and 4 generally showed the least reducing power and also the weakest reddish colorations with iodine in all of these experiments. The same was true of the materials in the alcoholic filtrates from the reprecipitated soluble starch of these two preparations. The acidity of each of these preparations (3 and 4), in terms of *n*/100 sodium hydroxide solution, was very slight. Preparation 2, however, had nearly as low an acidity as that of Preparations 3 and 4, but showed in almost every case, properties very much like those of Preparation 1, which had an acidity fifteen times as great as that of Preparation 2. It will be remembered that Preparations 1 and 2 were made from commercial potato starch. The process employed in the preparation of our specimen of the latter is unknown to us. That the method of preparing raw starch has some effect on the properties of the Lintner

⁹ The blank tests were negative in every case.

soluble starch produced from it is indicated by the differences to which allusion has just been made.

6. Comparative properties of Lintner soluble starch and soluble starch prepared with the aid of saliva. The preparation of Lintner soluble starch is an unusually tedious process. The final product is obviously a mixture of substances. It seemed probable that soluble starch, made through the almost instantaneous action of ptyalin or diastase and obtained in a comparatively pure form, would be as satisfactory for ordinary use as the Lintner product.

We prepared soluble starch for such a comparative study as follows: A thick paste was made by pouring 15 c.c. of cold water holding 4 grams of potato starch into a casserole containing 185 c.c. of water at 95° C., which was thoroughly stirred as mixture occurred. The fluid was promptly cooled to 40° C., then 5 c.c. of filtered saliva were added and the mixture thoroughly stirred. In two or three minutes the paste was completely liquified. The whole volume was then poured into one liter of 95 per cent. alcohol. All the soluble starch was apparently precipitated by this treatment, but to insure that result, a few drops of 10 per cent. sodium chloride solution were added. The soluble starch was filtered off and redissolved in 50 c.c. of water at 95° to destroy any contained ptyalin. Solution occurred immediately. The solution was cooled at once and reprecipitation in alcohol was promptly effected. The product was redissolved in cold water and reprecipitated several times in this way, and then dried in the open air before a fan.

Comparative tests of the responses to treatment with iodine solution, and reducing action upon the Fehling and Fehling-Benedict reagents yielded results which showed that this preparation of soluble starch was practically the same in these respects (and almost if not quite as pure) as all of our Lintner products. It was much more readily soluble than the Lintner products. The saving of time, energy and material in such a simple process, and the greater solubility of the resultant product, warrant preference for a method of this kind in the preparation of soluble starch for ordinary usage. The impurities which may be introduced with the enzyme are negligible in all but exceptional cases.

III. SUMMARY OF GENERAL CONCLUSIONS

The amount of reducing power of Lintner soluble starch, and the intensity of its erythrodestrin coloration with iodine solution, appear to be proportional, in a general way, to the *acidity* of the product.

By dialysis, aqueous solutions of Lintner soluble starch yielded to the diffusates considerable material possessing strongly marked reducing action and erythrodestrin properties. There was equivalent diminution in the proportions of reducing substances and erythrodestrin in the starch that remained in the undiffused residual liquid in these cases, though never complete removal of either.¹⁰

Our best preparations of Lintner soluble starch were made from potato starch of our own production. It is possible that the method of manufacturing the available commercial potato starch included an influence that was responsible for the difference noted.

The reducing power of a given preparation of Lintner soluble starch is largely, perhaps wholly, due to contained dextrans, from which the product can be purified only with the greatest difficulty, if at all. Consequently, it is not strictly justifiable to correct for this reducing power in diastatic determinations as Meyer¹¹ did, by subtracting the weight of cuprous oxide produced through the action of the soluble starch alone, from that of the cuprous oxide formed by the products of enzyme action upon the starch. Whether the original reduction in such cases is induced by traces of dextrans or not, it seems that this reduction represents material which may be *further* acted upon and removed by the enzyme, with the formation of maltose and other products of such action. Therefore, although the original reduction indicates the amount of reducing

¹⁰ These conclusions accord with the view of Brown and Morris (Journal of the Chemical Society, 1900, lv, p. 449), who consider that the reducing power of Lintner soluble starch is due to the presence of certain dextrans, which cannot be completely removed either by dialysis or precipitation.

Just as this work was nearing completion, it was discovered that Ford (Journal of the Society of Chemical Industry, 1900, xxiii, p. 415) had dialyzed Lintner soluble starch and had, in one or two details, anticipated our results. He stated that his dialysates contained material which gave a reddish brown coloration with iodine and which also possessed reducing power. Since his dialysates were evaporated at boiling temperature, it was impossible for Ford to say that this material was not produced by hydrolysis.

¹¹ Meyer: Loc. cit.

substance taken to begin with, it fails to represent that quantity of substance in the final reductions after the enzyme has altered it and perhaps removed it entirely.

In conclusion, the author wishes to acknowledge his indebtedness to Prof. William J. Gies for the many helpful and stimulating suggestions that have made this investigation possible.

ON MELANIN

ROSS AIKEN GORTNER

(Biochemical Laboratory of the Station for Experimental Evolution, The Carnegie Institution of Washington, Cold Spring Harbor, L. I.)

I. INTRODUCTION

Although the literature concerning the animal pigments is very extensive it is noteworthy that the work is not comparable owing to the great diversity of the methods by which the pigments were isolated. Great difference of opinion is also observed as to the ease with which melanins are decomposed, some authors claiming that boiling with concentrated acids does not alter their composition while others state that they are profoundly changed by the action of acids. The study of these pigments has, therefore, been taken up at this Station with the view of thoroughly investigating the nature of the melanin molecule and the reaction by which these pigments are formed in the animal body. The present paper is a report of some of the work which has already been completed.

II. THE CHEMISTRY OF MELANIN FORMATION

In 1896 Bertrand discovered a new type of oxidase which appears to be a true enzyme. He noted that this ferment was capable of oxidizing tyrosin through various colors to a black precipitate, and, therefore, gave to this type of oxidases the generic name of tyrosinase. Later, von Fürth (1902) summed up the instances where tyrosinases were known to occur in the animal kingdom and suggested that perhaps the melanins were the product of the interaction of this ferment and tyrosin. Almost coincident with the publication of von Fürth's theory confirmatory evidence was furnished by Dewitz (1902) who found that the pigmentation of the fly (*Lucilia Caesar*) was caused by the action of a tyrosinase on a chromogen. Roques (1900) showed that a similar action occurred during the metamorphoses and pigmentation of a beetle (*Limno-*

philus flavicornis Fabr.). Phisalix (1905) found that the color changes in the integuments of the cock-roach larva (*Phyllodromia germanica*) are produced in the same way.

Miss Durham (1904) obtained extracts from the fetal skins of rabbits and guinea pigs of black and agouti origin, which, when incubated at 37° for 10 days in the presence of tyrosin and one milligram of ferrous sulphate produced dark precipitates. When the skins of red guinea pigs were used a red precipitate was formed. Toluol was added as a preservative, and no coloration was produced in the absence of the ferrous sulphate. Although it is probable that the animal pigments are the result of oxidase action, only more extended research can show whether these colored precipitates were produced by the action of different tyrosinases or whether they were formed by some other reaction. It is obvious that the agency which produced the red pigment was different from that which caused the formation of the black precipitate.

I have found in a study of the pigment formation in the meal worm (*Tenebrio molitor*), the periodical cicada (*Tibicen septendecim* L.), and the Colorado potato beetle (*Leptinotarsa decemlineata* Say) that in each case the mode of pigment production is, in general, the same but that there are individual peculiarities which call for separate treatment.

Two varieties of tyrosinase occur in the meal worm, one variety being readily soluble in water and the other variety being characterized by its insolubility in aqueous media. The insoluble enzyme occurs in the larger quantity and is extremely active. When the larva sheds its skin the characteristic color pattern is wanting and the entire body appears creamy white. This nearly colorless appearance is retained after death, providing that the oxidase has been destroyed by heating to 70°–80° or else that the oxidation has been inhibited by keeping the larva in an atmosphere free from oxygen, or by immersion in water or oil. Subsequent exposure to atmospheric oxygen causes the normal color pattern to develop, providing that decomposition has not set in.

The Colorado potato beetle forms a color pattern in the same way in which the pigment is laid down in the integuments of the meal worm, with the exception that no evidence of the presence

of an insoluble tyrosinase was found. Soluble tyrosinase is, however, present in relatively large amounts. A possible explanation of the meaning of the color pattern in the elytra of this beetle has been found. If the elytra be removed before the color pattern develops (just after the beetle has emerged from the pupal shell) and floated on water exposed to the air, the normal color pattern is produced. If, however, the water is replaced by an aqueous solution of tyrosin the *entire elytrum* is colored black. It would thus appear that *the color pattern is caused by the localized secretion of chromogen* and that the secretion of the oxidizing enzyme is uniform over the entire elytrum.

In the periodical cicada, or seventeen-year locust, the mode of pigment formation is somewhat different. When the mature cicada emerges from the pupal shell the body is creamy white with the exception of the reddish eyes and two black spots on the prothorax. In a short time the creamy tint deepens and in a few hours the entire body is jet black. I was unable to find a tyrosinase in the body-filling of either the pupae or adult cicadas although an oxidase of some sort was present.¹ It was found however, that the outer surface of the body was moist and sticky, and by immersing the newly emerged adult in water, this moist, newly secreted, cuticula was dissolved and the solution was found to contain both a tyrosinase and a chromogen. It seems very probable that the tyrosinase is secreted and poured upon the surface of the body together with the new cuticula, and, indeed, it is not improbable that the entire new cuticula may be formed by the action of the tyrosinase on a chromogen. The same difference in solubility is observed between the newly secreted cuticula and the mature cuticula, as is found between the enzyme and chromogen, and the final pigment.

It would appear that in all cases where the origin of the pigment has been carefully investigated the production of melanin has been found to be due to the interaction of an oxidizing enzyme and an oxidizable chromogen, and it is very probable that all melanotic pigments originate in this manner. Even admitting the truth of this hypothesis several problems still remain to be solved: (1) The cause of albinism, (2) the sudden appearance of pigment in a color-

¹ Extracts of the body-filling oxidized a solution of guaiacol to an orange brown color.

less body, for example, the stimulus which causes the secretion of oxidase, or chromogen, or both, in the transformation of a colorless pupa to a pigmented adult, (3) the cause of those white colorations which are not albinic and which, when mated with colored individuals, produce white offspring in the first generation, and (4) the nature of tyrosinase, especially as to the cause of inactivation of some varieties of tyrosinase by precipitation with alcohol while tyrosinases of different origin are not affected by this treatment. It is possible that lack of enzyme, or chromogen, changes in permeability, and the presence of inhibiting factors may explain the first three problems but as yet definite data are wanting, and it is along these lines that future work will be continued.

III. MELANOTIC PIGMENTS

Methods of isolation. In a survey of the literature relating to the melanins I noticed a wide range in the methods by which the pigments were isolated. In some preparations the pigment was rubbed loose under water and, after washing with alcohol and ether, was called pure. In other instances the isolation was effected by boiling with 5-6 per cent. sodium hydroxide, or boiling with fuming hydrochloric acid. Indeed, instances were found where the keratin structure was destroyed by the action of cold, concentrated, nitric acid. In order to resist these reagents a substance should be almost as stable as the noble metals and, although one usually obtains a colored substance by any of these processes, it is highly improbable that the substance has been isolated in the form in which it occurs in nature. This is especially true of those "pigments" which have been isolated by the action of strong acids. Almost all of the proteins yield a black residue when hydrolized by strong mineral acids and even pure carbohydrates, such as cane sugar, yield a black residue when boiled with strong hydrochloric acid. It is very unfortunate that these black residues have been referred to as melanins, for there is no evidence that they bear the slightest relation to the melanin compounds, and until such a relation is shown they should not be confused with animal pigments. No such confusion would result if they were referred to under the name of *humins*.

In order to test the stability of the melanin molecule, I undertook the isolation of the melanin from black wool, and used varying strengths of sodium hydroxide solution to destroy the keratin structure, keeping all other factors as nearly uniform as possible. Experiments were carried out with solutions of sodium hydroxide of the strengths shown in Table I where the percent. of "pigment" found and its percentage composition are tabulated. For a detailed description of the methods employed the reader is referred to the original article; Gortner (1910), Studies on Melanin I.

TABLE I

Method	C	H	N	S	O (by diff.)	Yield of pigment
0.2 per cent. NaOH, soluble in acid	52.60	7.28	13.52	1.33	25.25	8.10%
0.2 per cent. NaOH, insol. in acid	53.44	5.81	10.44	1.16	29.15	1.36
1 per cent. NaOH.....	52.20	6.62	10.34	1.06	29.78	3.26
2.5 per cent. NaOH.....	53.07	5.82	9.37	1.06	30.68	2.95
5 per cent. NaOH.....	53.16	5.71	9.22	1.05	30.86	3.62
10 per cent. NaOH.....	56.01	4.88	7.03	1.24	30.84	2.43
20 per cent. NaOH.....	56.52	4.28	6.19	1.27	31.74	1.78
30 per cent. NaOH.....	56.71	4.30	5.12	1.46	32.41	1.71
50 per cent. NaOH.....	57.06	3.84	8.98	1.27	28.85	1.56
25 per cent. H ₂ SO ₄	57.81	4.40	5.50	1.75	30.52	2.00

It will be seen from this table that the use of strong alkali decomposes the melanin molecule, causing a loss of both nitrogen and hydrogen, and that one may obtain as many different products as is desired providing that the keratin is destroyed by different concentrations of alkali. It can readily be seen how different "melanins" have been isolated by different investigators using material of similar origin. Naturally the weaker concentrations of alkali cause the least decomposition so that in the future my work on melanin will be carried out with sodium hydroxide in 0.2 per cent. concentration.

The nature of melanin. The pigment preparations I have isolated were of two types. The greater portion of the product which was isolated by the action of 0.2 per cent. sodium hydroxide was soluble in dilute acids, while all of the other preparations were insoluble products. These insoluble products formed brownish black granules, powdering to a dark brown dust. They were insoluble in indifferent solvents and in dilute acids, soluble in concentrated

sulphuric acid, readily soluble when moist in alkalis, but when dried by heat, solution in alkalis took place only slowly.

The melanin which is soluble in dilute acids differs widely from the other preparations, and I believe it to be the pigment in more nearly the same form in which it occurs in the natural keratin. When freshly precipitated, the pigment is very plastic and may be moulded into compact balls. When heated on a water-bath, these balls contract strongly, forcing out water, and a tough, elastic mass results. When thoroughly dried, the product powders readily to a dark brown dust. When dried by heat, the pigment is found to be insoluble in dilute acids, and only incompletely soluble in alkaline solutions. After drying slowly, at room temperature, it completely dissolves in $n/20$ HCl or 50 per cent. acetic acid forming dense black solutions. Boiling with alkali, of a strength exceeding 0.2 per cent. sodium hydroxide, decomposes the melanin yielding, together with volatile and soluble products, a colored substance which is *insoluble* in acids.

By hydrolysis with strong mineral acids the melanin is decomposed, yielding a series of cleavage products, among which I have identified tyrosin, lysin, and arginin, and leaving a brownish black residue equal to about 10 per cent. of the original material. The nature of this "melano-humin" and the products of hydrolysis are being further investigated.

Judging from these data, *it appears that this melanin is of a protein nature. I believe it to be of the same type as an alkali albumin, but with a portion of the molecule so modified as to be deeply pigmented.* I have proposed for this type of compounds the name *melano-protein* when they are discussed under the proteins, and retain the name of *melanin* when they are treated under animal pigments, with the distinction, however, that *all melano-proteins are melanins. while only a portion of the melanins are melano-proteins.*

In the isolation of melanin from other keratin structures, by the method that was used in the case of the black wool, I have found melano-proteins in auburn human hair, and both light and dark brown horse hair. Brown human hair (three samples), black feathers from domestic poultry, negro hair, black rabbit hair, and crow feathers do not contain a melano-protein but give

pigments which are insoluble in dilute acids. Neither do these pigments correspond to the usual definition of melanin, i. e., soluble in alkalis and insoluble in acids, for they are *insoluble* in dilute alkali and only by prolonged boiling with 0.2 per cent. sodium hydroxide do some dissolve, while others resist this treatment and do not go into solution until a higher concentration of the alkali is employed. Apparently the melanin that occurs in the keratin structure is, in these cases, insoluble in alkaline media and *only after a certain amount of decomposition has taken place does solution occur.*

There are, therefore, at least two types of melanin and these occur in three combinations. In only one instance (auburn human hair) have I found only the melano-protein. In black wool and in pigmented horse hair both the melano-protein and an acid-insoluble melanin are found, while in other keratin structures (see above) only the acid-insoluble pigment is present.

As to the cause of these differences, no data are, as yet, available. It appears, however, very probable that we shall have to go back to a modified form of a theory which has been long abandoned and postulate that, in at least some instances, the melanin is *dissolved* in the keratin or rather that *a portion of the protein structure has been so altered as to become deeply pigmented.* Even if we grant that the melanins are the product of an oxidase action, in no case do we know the exact nature of the chromogen involved. In some cases this chromogen may be relatively simple, and it may, indeed, be only tyrosin, but there is no reason why this chromogen should not be very complicated in other instances, even consisting of an entire protein molecule. This being the case we should expect to be able to isolate pigments which are a part of a protein residue, and such pigments we find in the melano-proteins. These pigments should not appear as granules, but rather as a diffused coloration of the keratin *and such a diffuse coloration is found in the case of auburn human hair* where no granules are to be observed (Davenport, 1909). The acid-insoluble pigments, on the other hand, represent the pigment granules, which may be formed either by the oxidation of a second chromogen, or by a further oxidation of the melano-protein, whereby the pigmented portion of the molecule is retained and broken off from the protein residue. To my mind the

hypothesis of a second chromogen appears the more probable, inasmuch as in a cross between red haired (no granules) and dark haired individuals (granules present) all of the offspring are dark haired, and granules appear in the hair *but the diffuse red coloration is also present* being masked by the darker pigment (see Davenport).

IV. CONCLUSIONS

1. All available data indicate that the formation of melanin is brought about by the interaction of an oxidase and an oxidizable chromogen.

2. Melanins are of at least two types which may be differentiated by their solubility or insolubility in dilute acids.

3. Those melanins which are soluble in dilute acids are of a protein nature and for this type the name melano-protein is suggested. It appears probable that these melano-proteins are not present as granules, but that they are "dissolved" in the keratin structure.

4. The melanins which are insoluble in dilute acids are of an unknown constitution, and are, probably, the "pigment granules" which may be seen in the hair and tissues. It is probable that they are formed by the oxidation of a different chromogen from that which yields the melano-proteins.

5. The protein portion of the melano-protein molecule is readily decomposed by the action of alkalis or acids, and colored products are obtained which are not soluble in dilute acids.

6. Tyrosin, lysin and arginin have been identified among the hydrolytic products of a melano-protein.

7. Sodium hydroxide solution decomposes the melanin molecule, and causes a loss of both nitrogen and hydrogen. As many different products as may be desired, can be obtained by varying the strength of the alkali employed.

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CHEMICAL NOTES ON THE EGG CAPSULES OF TWO SPECIES OF SHARKS

LOUIS HUSSAKOF AND WILLIAM H. WELKER

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York)

(With plate 4)

I. INTRODUCTION

Various types of sharks deposit eggs which remain in the water for a considerable time—in some forms for nearly a year—until the young are hatched and capable of independent existence. These eggs are provided with very resistant egg-shells, or “egg capsules,” composed of material capable of withstanding water action for a long period. The capsules consist of an elastic shell, yellowish-brown, reddish-brown, or chocolate in color, semi-translucent, and somewhat horn-like in appearance. The form of egg capsule varies with the species; one of the commonest is that shown in figure 1, and a rarer form is that shown in figure 2.

The chemical composition of these egg capsules has apparently never been investigated. We have been unable to find any chemical references to them. Superficially their substance is suggestive of keratin; but the tests which we have made, as shown in this paper, indicate that this substance is distinct from keratin.

The observations which we desire to record in this preliminary communication have to do with both the egg capsules and the coloring matter which they contain.

II. MATERIAL STUDIED

We employed the egg capsules of two very different sharks: (1) the skate (*Raja erinacea*) and (2) the Port Jackson shark (*Heterodontus philippi*). The former species is quite common on the north shore of Long Island, where its empty, blackened and weatherbeaten capsules are washed ashore with other detritus. A

number of these egg capsules were collected for this study. Another supply which we employed was taken directly from freshly caught skates. The capsules were freed from their contents through a longitudinal slit in each.¹

The egg capsules of the Port Jackson shark were sent us from Japan in broken and dried condition.²

III. PREPARATION OF MATERIAL

Egg capsules, especially such as are taken directly from the fish, usually have flakes of dried ovo-protein adherent to their inner sides. This material must be entirely removed, to insure an accurate chemical study of the capsule. Our method of doing this was practically the same with both kinds of capsules and was essentially the following:

The capsules were cut into small pieces and thoroughly washed in running water. The pieces slowly sank to the bottom of the vessel, allowing the lighter membranous films, which remained on top, to be removed by simple decantation. Repeated washings also served to dissolve and carry away some of the substance adherent to the true capsular material. The washed pieces were then spread out on sheets of white paper and all visible bits of adherent material picked off by hand.

A preliminary test proved that peptic digestion, even when continued for 24 to 36 hours, does not visibly affect the capsular substance. The capsule pieces, after several additional washings in water, were repeatedly subjected to long periods of peptic digestion, until digestible protein had been entirely removed. They were likewise subjected to pancreatic digestion, but in this case the resultant change was inconsiderable: the digestive fluid became slightly yellowish. The pieces were then washed successively in water, alcohol, and ether, and finally dried over sulfuric acid. All this preparatory treatment failed to affect the capsular substance

¹For these, as well as for a supply of sun-dried *Raja* capsules, picked from the beach, we are indebted to Dr. Francis B. Sumner, Director of the U. S. Fish Commission Laboratories at Woods Hole, Mass.

²For this rare material our thanks are due to Professor Bashford Dean, of Columbia University, who forwarded it to us from Japan, where he was at that time (1905) carrying on biological investigations at the Misaki laboratory.

to any visible degree. The pieces were then ground to a fine powder. This was an exceedingly difficult task, owing to the toughness of the material. The powder was grayish-black in color for the skate capsules, and reddish-brown for the Port Jackson shark capsules.

IV. SOLUBILITIES OF THE CAPSULAR SUBSTANCE

The solubilities of the predominant material in both the *Raja* and the *Heterodontus* capsules are given in the following table.

Solubilities of the keratinoid substance in the egg capsules of Raja erinacea and of Heterodontus philippi.

Reagent	<i>Raja</i>		<i>Heterodontus</i>	
	Cold	Boiling	Cold	Boiling
Water.....	Insoluble	Insoluble	Insoluble	Insoluble
Alcohol, 95 per cent.....	Insoluble	Insoluble	Insoluble	Insoluble
Chloroform.....	Insoluble	Insoluble	Insoluble	Insoluble
Ether.....	Insoluble	Insoluble	Insoluble	Insoluble
Sodium carbonate, 0.5 per cent.....	Slightly soluble ³	Slightly soluble ⁴	Insoluble	Slightly soluble
Potassium hydroxide, 0.1 per cent.....	Soluble ⁵	Soluble	Insoluble	Insoluble
Potassium hydroxide, 5 per cent.....	Soluble	Soluble	Slightly soluble on standing	Insoluble at first, some- what soluble if boiling is continued
Potassium hydroxide, 10 per cent.....	Soluble	Soluble ⁶	Slightly soluble on standing	Insoluble at first, some- what soluble if boiling is continued
Hydrochloric acid, 1 per cent.....	Insoluble	Slightly soluble	Insoluble	Insoluble
Hydrochloric acid, 10 per cent.....	Slightly soluble	Soluble ⁷	Insoluble	Soluble

From this table it is seen that the substance of both capsules is very resistant to the action of ordinary solvents. The powdered materials were insoluble in water, alcohol, chloroform and ether. They were soluble in both acid and alkaline solvents. The substance of the skate capsule was more soluble in alkaline than in

³ Slightly more soluble than in cold 10 per cent. hydrochloric acid solution.

⁴ More soluble than in 1 per cent. hydrochloric acid solution.

⁵ Dissolved completely on long standing.

⁶ Samples of both the powdered and the whole capsules dissolved on boiling, in twenty minutes, giving a deep wine red colored liquid.

⁷ Much less soluble than in potassium hydroxide solution. The solution had a wine red color by reflected light.

acid media, whereas that of the Port Jackson shark capsule, while more resistant to both types of reagents, was somewhat more soluble in strong acid than in concentrated alkaline media.

V. GENERAL QUALITATIVE TESTS

Both types of capsules gave the same general reactions when the powdered substance as well as the washed and dried fragments of the original material were tested. These tests showed the presence of sulfur⁸ and nitrogen,⁹ and the absence (or presence in exceedingly slight proportions) of phosphate.¹⁰ Hydrolysis with boiling dilute hydrochloric acid for 30 minutes failed to yield sulfate or a reducing substance. The capsular material did not respond to the Molisch test for carbohydrate, but gave the typical colorations in the general protein color tests.

VI. PIGMENTS

Pigments from egg capsules of the skate. About five capsules were completely dissolved in boiling 5 per cent. potassium hydroxide solution, by which process a deep wine-red fluid was produced. This was filtered and the filtrate carefully neutralized with hydrochloric acid. A heavy, flocculent, gray precipitate resulted, which was filtered off.¹¹ The filtrate containing the coloring matter was then spontaneously evaporated to dryness in a warm place. When dry, a concentrated aqueous solution was made of the residue. This solution was placed in a parchment bag and dialyzed. The coloring matter did not pass through the bag and after several days' dialysis, the colored solution was obtained free from salts. The solution was again filtered and evaporated to dryness as before. The residue was a shiny, translucent, scaly, reddish-black solid.

Solubility tests applied to these scales showed them to be soluble in water, acid and alkali. Solutions of this pigment are delicately responsive to change in reaction; when acid, the solutions are straw colored; when alkaline or neutral, they are dark brown.

⁸ The material was fused with metallic sodium and the aqueous solution of the residue was tested with sodium nitroprusside.

⁹ Determined with the aid of the Lassaigne test.

¹⁰ The material was decomposed with nitric acid and the resultant solution was tested with molybdate solution.

¹¹ The further treatment of this precipitate is described on page 220.

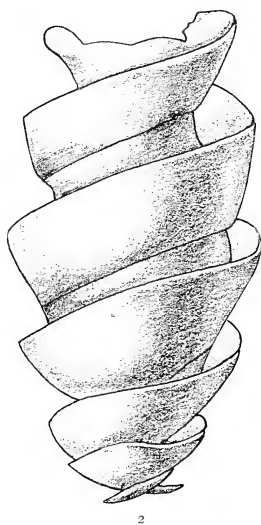
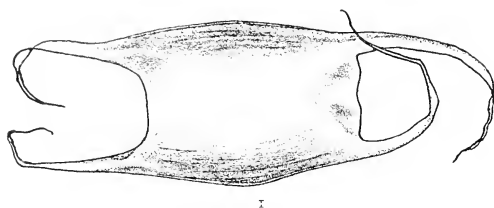
Pigments from egg capsules of the Port Jackson shark. The coloring matter of the *Heterodontus* egg capsules was prepared in a manner similar to that employed for the isolation of the *Raja* product, the only difference being that a 10 per cent. hydrochloric acid solution was employed, instead of a 5 per cent. potassium hydroxide solution. The acid medium was carefully neutralized with potassium hydroxide. The neutralization precipitate was filtered off¹² and the filtrate dialyzed. The salt-free solution was evaporated spontaneously to dryness.

The pigment scales thus obtained were almost colorless. They were soluble in water, in alkali, and in acid (more readily than in alkali). In 10 per cent. hydrochloric acid solution, the pigment yields a solution, which, on shaking, becomes frothy like an aqueous protein solution. This solution is light orange in color. The color of the neutral solution of this pigment is similar to that of the acid solution. The alkaline solution is brownish-yellow in color. The solutions of this pigment are much less delicately responsive to changes in the reaction, than are those of the pigment from the skate capsules. The pigment is insoluble in both hot and cold alcohol (95 per cent.), and in ether and chloroform.

Each of the coloring matters of these capsules gave evidence of the presence of nitrogen by the Lassaigne test. The xanthoproteic, Millon, and Hopkins-Cole tests were positive with both of the coloring materials. The colored solution interfered with the biuret test in such a way as to make the result doubtful.

On the nature of the neutralization precipitate from the pigment extracts. Neutralization of the pigmentary extracts of the capsular material yielded, in each case, a grayish flocculent precipitate which was isolated by filtration. The precipitate was washed with water and redissolved in 5 per cent. potassium hydroxide solution. The solution was filtered and then neutralized. The reprecipitated matter was again filtered off and the precipitate thoroughly washed. The precipitate in each case responded positively to the Millon, xanthoproteic and Hopkins-Cole tests. That this substance is protein, in whole or in part, is obvious. It appears to be metaprotein.

¹² The further treatment of this precipitate is described under the next sub-heading.



VII. SUMMARY OF GENERAL CONCLUSIONS

The egg capsules of the skate and the Port Jackson shark consist, in the main, of materials that resemble keratin superficially, but which differ in essential respects from that substance.

The keratinoid substances obtained from the two types of egg capsules are alike in some respects, e. g., in their insolubility in water, alcohol, ether and chloroform. They are different, however, in other regards. Differences were observed in their behavior in alkaline and acid media, the product from the *Raja* capsules being somewhat more soluble in alkaline reagents, that from *Heterodontus* was more soluble in concentrated acid media.

It is obvious that this keratinoid material imparts to the egg capsules the mechanical protective qualities which are characteristic of them.

The egg capsules of these fishes contain pigments which, in alkaline solution, are dark red in color. Though somewhat different in tinctorial effects, the coloring matters bear close resemblance to each other, as was shown by their similar behavior in various qualitative tests. Possibly they are as nearly the same chemically as bilirubin and biliverdin.

Neither the exact chemical nature of the pigments, nor their functional significance, has as yet been determined. That the pigments are melanins seems probable.

The writers are deeply indebted to Prof. William J. Gies for much valuable advice given them during the progress of this investigation.

VIII. EXPLANATION OF FIGURES

FIG. 1.—Egg capsule of the Skate (*Raja erinacea*); natural size. Long Island.

FIG. 2.—Egg capsule of the Port Jackson Shark (*Heterodontus philippi*); $\times \frac{1}{2}$. Japan. (After Waite.)

A CAGE FOR METABOLISM EXPERIMENTS ON GOATS

A. R. ROSE

(New York Agricultural Experiment Station, Geneva, New York)

(With Plate 5)

One of the first essentials in metabolism work is the provision of known conditions under which true samples are always obtainable. When animals are used as subjects, much ingenuity has to be exercised to secure such conditions. One of the most satisfactory ways is to use carefully devised cages. These are, however, impractical in the case of so large an animal as the cow. Sometimes harnesses have been employed to hold suitable receptacles for excreta in place upon such animals, but methods of this character are troublesome and inadequate. In the New York Agricultural Experiment Station it was found most feasible, when cows were used in metabolism experiments, to keep men constantly on the watch to collect the urine and dung in large receptacles made for the purpose. This method is quite laborious and expensive, however, and led to consideration of the use of some animal which could be more easily caged than the cow, and would serve similar purposes in metabolism investigations.

The goat was chosen. It seems strange that an animal with so many qualifications for this kind of work has received so little attention in this connection. It is hardy by nature; of convenient size to be easily handled; takes rations and yields excreta of very satisfactory bulk. It may well represent the herbivora in animal experimentation, just as the dog does the carnivora. It has the advantage over the rabbit, so frequently selected as a typical herbivorous animal, in that it lends itself to problems involving a study of lactation; and over the cow usually employed in such cases, whenever the problem requires an expensive ingredient in the ration, since the food consumption of the goat is only about one tenth of

the amount required by the cow. In cases of this kind, considerable care must be exercised in choosing the subject and adjusting her to the experimental rations, because the goat, like the cow, frequently loses appetite under unusual conditions. Aside from this, the goat is no more irritated by confinement than the dog and, unlike the cow, does not require bedding.

The dog cage has been developed into an admirable piece of apparatus but, not having been designed for the goat, would not be suitable for quantitative work on the goat because of the differences in bulk and friability of the feces of these two types of animals. A special cage was, therefore, designed to meet the requirements of the work at this Station, where goats are used in a study of phytin metabolism. These requirements seemed to be:

1. Reasonable comfort for the animal.
2. Adequate feeding with a minimum amount of waste.
3. Complete removal of urine and feces from the cage.
4. Separation of the urine and feces without contamination of one by the other.
5. Easy access for milking.

The cage to be described has fulfilled the above conditions. It consists essentially of an elevated wooden box, with side gratings in the upper part to admit light and air. The major portion of one side swings from the top by means of hinges, and thus forms a door (*I*) for the admittance and removal of the goat, and for convenience in milking. The goat is tied by a chain attached to the side wall in the front of the cage, to keep her from turning around. The floor consists of a screen made of heavy wires sufficiently far apart to let all waste pass through, without the hoofs of the animal being caught in the meshes. Under this screen, at the front end, is a pan to collect any food that may be dropped in eating. Under the rest of the floor is a device for separating urine and feces, made of galvanized sheet iron and comprising two parts, the hopper and the urine pan, which connect with separate receptacles on the floor.

The cage is simple in construction. It was made by local carpenters with the aid of a tinsmith, at a cost of \$37.00; occupies a floor space of two by four feet, stands seven feet high, and can be easily carried by two men. Four pieces of 2" \times 4" pine constitute

the supports, which elevate the box forty inches from the floor, and also serve as the upright corner posts of the framework of this box, whose inside dimensions, exclusive of the space over the feed box, is forty-eight inches long, twenty inches wide, and thirty-four inches high. The space for the feed box extends the cage box forward another twelve inches. The sides, not including the twelve-inch space allowed for the feed box, are made of two parts, the lower twenty inches being of boards (*G*), and the space above (*A*) is fitted with quarter-inch iron rods placed perpendicularly three inches apart. The rear wall, roof, and the portion of the walls above the feed box, are covered with boards. All inside exposures, for twenty inches from the bottom of this box, are protected by galvanized sheet iron (*C*), and a triangular wooden strip (*D*) is nailed around the side walls just above the bottom, over which the galvanized iron is bent, to direct all drippings from the sides of the cage into the hopper.

Seven inches above the screen floor, at the front of the cage, is the removable feed box (*G*), eighteen inches long, ten inches wide, and seven inches deep (inside measurements). This box is lined with sheet iron, which extends as a facing over the outer portion on the side next to the goat. A wall (*H*) of the same materials extends from this side of the feed box to the floor, partitioning off a space from the rest of the cage, in which utensils may be kept.

The screen floor is specially woven from No. 10 iron wire, over a frame of quarter-inch iron rod, with the lengthwise strands five eighths of an inch apart; the crosswise, seven eighths of an inch apart. The whole is afterwards galvanized. This floor slides in or out through an opening (*J*) in the rear end of the cage, just large enough to let the floor pass through, permitting an easy and quick exchange of screens when desirable. A screen of different mesh may be convenient to have on hand, if the dung pellets are unusually large, or become soft and tend to cling together.

The pan (*K*), to catch any fodder that may be dropped in eating, slides under the floor screen from the side, and covers the space between the feed box and the hopper, which extends under the rest of the screen, a space of thirty by twenty-one inches. Except in front, the upper edges of the hopper are fastened to a wooden frame

(*L*) thirty inches long, twenty-one inches wide, and eighteen inches high. This frame slides readily on two strips of lumber (2" \times 4" pine) nailed to the inner sides of the legs twenty-seven inches from the floor, so that the hopper is easily accessible for cleaning. These strips are long enough to extend about twenty inches beyond the legs, front and back, so as to form convenient handles for lifting and carrying the cage.

The hopper (*M*), eighteen inches deep and narrowing to an opening eight by ten inches, joins a trough (*N*), the bottom of which is in a continuous line with the rear side of the hopper. This trough is eight inches wide at the bottom, and fourteen inches wide at the top for the first eighteen inches, and then tapers gradually for sixteen inches, to a semicircular open end, three inches in diameter. The walls of the trough are soldered firmly to the sides of the hopper. Adjoining the hopper, the bottom of the trough is cut away for the first eighteen inches, and into this opening are soldered twenty-six parallel wires, fastened to the bottom of the trough at one end, and to the lower edge of the rear side of the hopper at the other. Six inches from each end of the wires, a narrow strip of sheet iron is soldered across them, to keep them rigid. The dung pellets roll down the trough over the grating thus formed in the bottom of the trough, but the urine flows through into the pan (*P*) beneath, and the drops running down the wires are arrested by the crosswise strips and directed into the pan. The trough is supplied with a removable cover extending beyond the lower end and at that point is bent downward, to prevent the pellets from bounding out and scattering over the floor.

The urine pan (*P*) is held in place directly under the opening of the trough by means of hooks and eyes, so as to prevent loss by spattering. The pan is two inches deep, and in the upper part, under the opening, is ten inches wide. Below this, the sides converge to form a spout with an open end two by two inches.

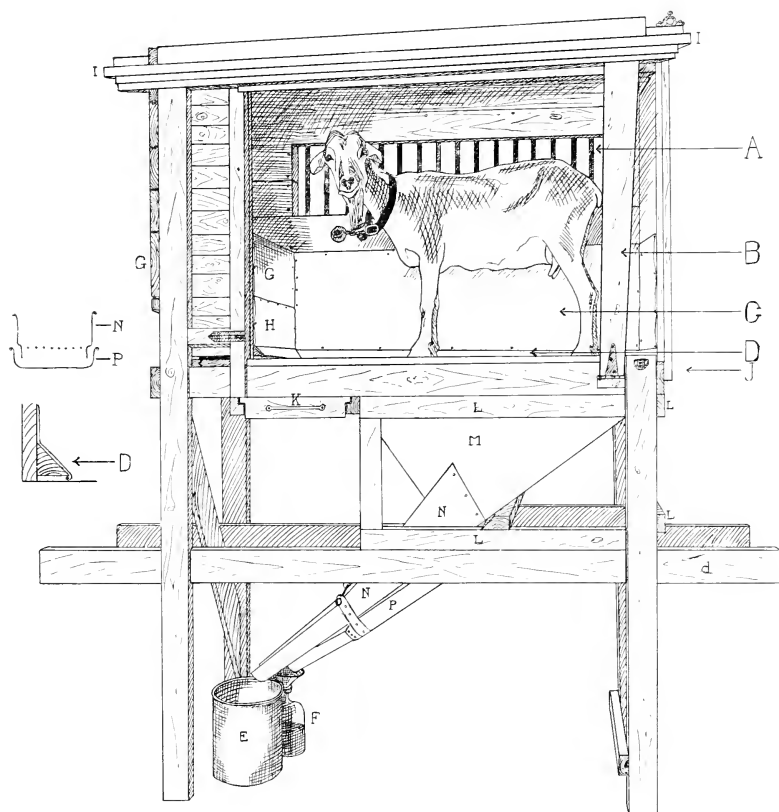
The urine receptacle (*F*) stands on the floor beside the one provided for the dung (*E*), and in order that the spout may discharge into it, a slight bend to the right is made in the urine pan twenty-two inches from its upper edge.

While the cage herein described may seem crude in some

respects, it has served its purpose. It is to be hoped that other investigators will try the goat in studies of their metabolism problems. We may then expect developments both in the construction of the cage, and in the manner of conducting experiments with goats, leading to the perfection of technic which we now have in experiments on dog metabolism. Such a piece of apparatus as a metabolism cage is necessarily the product of evolution along lines suggested by the investigator's experience. To Professor Gies we are greatly indebted for the perfection of technic in experimentation on dog metabolism. He has given us the first published detailed description of a dog cage suitable for accurate quantitative work. His improved device, as described in 1905, in the *American Journal of Physiology*, Volume XIV, page 403, was the suggestion from which this goat cage resulted.

EXPLANATION OF PLATE V.

(See description on pages 223-225.)



Drawn from photo by W.J. Schoenp

W.J. Strand
1911

ROSE: A CAGE FOR METABOLISM EXPERIMENTS ON GOATS.

THE PERMEABILITY OF CELLS FOR DYES AND ALKALIES¹

E. NEWTON HARVEY

(Zoölogical Laboratory, Columbia University)

Of the many methods employed by various investigators in the study of cell permeability, by far the most reliable are those which make use of some microchemical test to determine the penetration of the substance in question. The chemical reagent may normally occur within the cell, or, if harmless, may be introduced from without to serve as an indicator. Pfeffer made use of the tannin compounds occurring in the sap vacuoles of such algæ as *Spirogyra* to study the absorption of aniline dyes, while Overton also employed *Spirogyra* in studying the penetration of the alkaloids and organic alkalies. The tannin is precipitated, forming a tannate. Plant cells often contain natural pigments, anthocyan compounds, which may serve, by their color changes, as indicators of the penetration of all the alkalies. Both Pfeffer and de Vries have studied the permeability of such pigmented cells for ammonium hydroxid, potassium hydroxid, sodium hydroxid and also for acids.

I have investigated the permeability relations of four types of cells (those of *Elodea* leaves, *Spirogyra* filaments, *Paramæcium* and the eggs of various Echinoderms) for organic and inorganic alkalies. The dye, neutral red, was introduced to serve as an indicator for alkali. The color change in water from red (in neutral and acid solution) to yellow (in alkaline solutions) occurs in an H ion concentration of 1.10^{-7} to 1.10^{-8} . Within the cell the dye unites with certain constituents, forming red compounds, precipitates or non-diosmotic substances. Hence a continuous accumulation takes place and the dye compound becomes clearly visible even in a space of one cell diameter.

¹ The original paper of which the following is an abstract, appeared in the *Journal of Experimental Zoölogy*, 1911, x, p. 507.

The composition of the dye compounds is different with each type of cell studied. In *Spirogyra* neutral red is precipitated by tannin as a tannate; in *Elodea*, a soluble red compound is formed in the cell sap, probably with some organic acid; in *Paramæcium*, colorless granules of unknown composition are stained red, while in marine eggs the granules which stain (as a rule the heaviest of the visible substances present) are possibly lecitho-protein in nature. Before the yellow color appears in alkali the above mentioned compounds must be broken up and the free dye base liberated.

Studies of the dye combinations formed in various plant and animal cells have revealed the interesting fact that neutral red as well as many other basic dyes, fails to enter cells in the presence of very weak acid (too weak to harm the cells), *i. e.*, in the acid or dye salt condition. Only the free dye base can penetrate the plasma membrane of living cells. A small amount of the base is formed in neutral distilled water, through hydrolytic dissociation, and it is found that cells stain readily in distilled water. Methylene blue, saffranin, methyl violet, Bismark brown, thionin, chrysoidin and toluidin blue behave as does neutral red. On the other hand, the acid dyes, eosin, Bordeaux red, säureviolett and aurantia cannot penetrate in neutral or slightly alkaline condition (as the dye salt) but do enter in acid condition (as free color-acid), staining and killing the cell. Salts are highly ionized in solution whereas weak bases and weak acids (such as the free color-bases and color-acids) are not. The above relation among dye compounds as well as a similar one discovered by Overton for the alkaloids, together with the observations described below on various alkalies, all point to the conclusion that cells offer great resistance to the entrance of the ions of a substance, but very little to the undissociated molecule.

Whether this is a primary relation or depends on the lipoid-solubility of un-ionized and the lipoid-insolubility of ionized substances, as Overton has maintained, is not yet definitely settled. According to Robertson, it is the free color bases and acids and not their salts which dissolve readily in fatty and fat-dissolving substances like ethyl acetate. Such are, in a general way but with many exceptions, the permeability relations of cells for dyes.

Mathews, on the other hand, demonstrated that the acid dyes form combinations with proteins in acid solution, the basic dyes in alkaline solution; a statement true also for the stainability of the lecith-albumin yolk platelets of the frog egg, as I have determined. It is therefore conceivable that a dye must combine with the surface layer of a cell in order to enter, a theory which Mathews has recently upheld.

Three complications arise from the use of an indicator to detect the entrance of alkali. First, the neutral red is not in watery solution but, as already mentioned, in combined form within the cell and the combination must be decomposed by the alkali. Second, neutral red, as I have determined by experiment, even in solution, cannot detect, in the presence of proteins like egg albumen, a certain amount of added alkali which combines with the albumen. Third, an acid may be secreted by the cell which neutralizes entering alkali. Such a secretion is actually formed by red stained *Elodea* cells when treated with chloroform water, if a deepening in color of the red dye is to be interpreted as the result of acid formation.

The above three objections may be met by comparing the rate of entrance of the alkali into similar living and dead cells, the latter serving as controls to determine the resistance, for there is always a resistance—although slight for some alkalies—offered by the normal cells. Thus the concentration of alkali which brings about instantaneous decomposition of the indicator in chloroform-killed cells can be determined. It can likewise be shown that the great impermeability of living plant and animal cells for the inorganic alkalies is an actual impermeability, dependent on the composition and properties of the cell surface and not an apparent one due to the time consumed by alkali in its decomposition of indicator compound, or in its combination with cell protein or in its neutralization of acid secretion. Chloroform saturated water has no effect on the combining power of egg albumen with either sodium hydroxid or ammonium hydroxid.

As regards penetrating power two classes of alkalies may be recognized, the strong [including Na, K, Ca, Sr, Ba and $N(C_2H_5)_4$ hydroxids] and the weak (including NH_4OH , methyl, dimethyl,

trimethyl, ethyl, propyl and isopropyl amines). All the cells thus far tested are very little more permeable to the latter group when dead than when living. Practically no resistance is encountered at the cell surface. With red stained *Elodea* leaves, for instance, the color change takes place in 1-2 minutes in $n/40$ concentration. With the strong inorganic alkalies on the other hand, from about fifteen minutes for the penetration of $n/40$ $\text{Ba}(\text{OH})_2$ to twenty-five minutes for $n/40$ NaOH is required. All the strong alkalies penetrate similar leaves of *Elodea* in less than one minute when killed in various ways; by heat, by chloroform, by HCl or by HgCl_2 . Such a post-mortem increase in permeability has been described by many investigators for other classes of substances and hardly requires special emphasis for the inorganic hydroxides except as showing the degree of impermeability of the normal surface.

Elodea leaves which have been "decolorized" by Na , K , Ca , Sr or $\text{N}(\text{C}_2\text{H}_5)_4\text{OH}$ and then immediately placed in fresh water, never recover and the neutral red never becomes red again. If decolorized with ammonia and the amines, the cells not only become red in pure water but no irreversible effects are produced by the action of NH_4OH or trimethyl amine at all, although methyl, dimethyl, ethyl, propyl and isopropyl amines generally kill the cells. Death of the cell is thus not necessarily connected with the intracellular presence of a certain concentration of OH ions, and the saturation of cell proteins with NH_4OH appears to be possible without detriment to the cell. The opposite is the case with NaOH .

Making use of the facts described in the last paragraph we can readily conduct a striking demonstration of the characteristic permeability relations of *Elodea* for NaOH (representing the strong alkalies) and NH_4OH (representing the weak), an excellent example of selective permeability. A leaf is "decolorized" in $n/50$ NH_4OH and then placed in $n/50$ NaOH . Although the indicator is already in the alkaline condition, and the cell proteins are saturated with NH_4OH , and the leaf cells are surrounded with $n/50$ NaOH , not enough NaOH enters the leaf to maintain the yellow color of the dye, but the whole leaf becomes red again in NaOH , from the diffusing away of the NH_4OH . Only after the usual 25-35 minutes does the NaOH break through the resistance offered by the surface and decolorize the leaf.

Despite the fact that the group of strong alkalies encounter a marked barrier at the surface of the cell and, in fact, fail to enter before fundamentally modifying the nature of the surface, they nevertheless produce changes in the normal functional activity of the cell *without entering*. When *Paramœcium* is placed in any alkali a certain series of changes ensues. Usually the avoiding reaction is given, followed by a change in shape, swimming backward, very slow swimming, the appearance of clear droplets at the surface, the fusion of the droplets to a more or less clearly defined membrane with cessation of movement and finally the bursting of the motionless and much distorted organism. Only after all motion has ceased and the *Paramœcium* is formless and dead, does NaOH pass in and convert the red granules to yellow ones. The decolorization, when once started at the surface, rapidly progresses to the interior as the alkali enters. Other strong alkalies [K, Ca, Ba, Sr and $N(C_2H_5)_4OH$] behave as does NaOH. NH_4OH and the amines, on the other hand, begin to diffuse inward from the moment the organisms are placed in the solutions and the latter become completely colorless before the clear droplets appear at the surface. The difference in permeability cannot be explained by a difference in combining power of NaOH and NH_4OH but must be referred to an influence of the NaOH on the *surface* of the cell independently of any action on the cell interior.

Protoplasmic rotation in the leaf cells of *Elodea* ceases long before enough NaOH has entered to turn the neutral red yellow, and irregular fragmentation and division of echinoderm eggs may also be induced without the entrance of NaOH.² Such a surface change may be in three conceivable directions: surface tension, electrical polarization and permeability. The alteration in the latter property is in the direction of an increase, since finally the inorganic alkali

² Warburg (*Zeit. für physiol. Chemie*, 1910, lxvi, p. 305) in a paper on oxidation, which I have but recently read, since its title gave no hint that the question of permeability was discussed, showed that NaOH fails to enter sea urchin eggs stained in neutral red whereas NH_4OH does, and further that neutral red is a more delicate test for the presence of OH ions than increased rate of oxidation. In sea water plus NH_4OH the red dye is affected, yet oxidations in the eggs are hardly increased over the rate of oxidation of eggs in neutral van't Hoff's solution. In sea water plus NaOH the rate of oxidation is very markedly increased.

itself may enter. The changes undergone by *Paramæcium* or *Elodea* or the sea urchin egg in NaOH must be the expression of some profound alteration of the cell surface involving one or all of the properties mentioned above. Functional changes cannot, therefore, be used as a criterion of permeability.

The normal resistance of the surface of *Elodea* cells and sea urchin eggs for NaOH can be diminished by the addition to the NaOH of small concentrations of ether or chloroform, too small to produce any irreversible changes in the absence of the NaOH. Ether and chloroform induce the formation of an artificial fertilization membrane about sea urchin eggs, but the amounts used in testing their effects on the surface were too small to cause membrane formation, although sufficiently great to inhibit the protoplasmic rotation characteristic of *Elodea* cells. Neutral salts (NaCl, KCl and CaCl_2) also increase enormously the rate at which the alkali enters *Elodea*. Nevertheless the protoplasmic rotation may continue for hours in $m/10$ NaCl. Thus, the modification of the cell surface by NaCl is not correlated with a change in functional activity (protoplasmic rotation), as is the modification produced by ether or chloroform. In some cases, namely in the echinoderm egg, a change in activity is associated with a normal change in permeability. Just after sperm fertilization or artificial fertilization the surface is much less resistant to NaOH than is that of the unfertilized egg. Evidence has recently been accumulated by several observers which tends to show that the fertilized egg is more permeable for various substances than the unfertilized, and it is possible that an increase in permeability may be the factor directly initiating development.

The presence of traces of salts has a relatively great influence in determining the properties of the cell surface. Thus, $n/40$ NaOH dissolved in pure redistilled water (quite harmless for *Paramæcia*, a sufficient test of the purity of a water) enters *Elodea* leaves in 25–30 minutes but the same $n/40$ NaOH dissolved in tap water requires about 45 minutes to enter. The small proportion of salts in tap water evidently confers on the cell membrane quite different properties from those it would possess in the absence of salts, *i. e.*, in distilled water. The resistance of many

organisms, especially *Spirogyra*, to NaOH or KOH varies considerably from day to day and this variation is possibly correlated with the accumulation of excretory products or of traces of other toxic substances in the water.

The toxicity of all the alkalis studied bears no relation to the degree of dissociation. The order of toxicity for *Paramæcium* is $N(C_2H_5)_4$ (?)³ < Na, K, Ca < Sr, Ba < NH_4 < amines. The order of dissociation is NH_4 < $N(CH_3)_3$ H < primary and secondary amines < $N(C_2H_5)_4$ < Sr, Ba, Ca, Na, K. This may in large part be understood when we consider that the weak alkalis enter the cell readily and the strong do not. But even among the strong alkalis, Ba and Na, about equally dissociated, the Ba is much more toxic.

The order of penetration rate for *Elodea* [NH_4OH more rapidly than primary and secondary amines > $NH(CH_3)_3OH$ > Ba, Sr > Ca, K, Na, $N(C_2H_5)_4OH$] is not the order of diffusion in pure water. Those strong alkalis which most readily affect the cell surface enter most rapidly, and are most toxic. But there is no direct relation between toxicity and penetrating power since the strong alkalis bring about many detrimental changes without entering the cell.

Considering the weak alkalis alone, NH_4OH enters *Elodea* most rapidly, yet is least toxic. Trimethyl amine is less dissociated than the primary and secondary amines, yet enters the cell less rapidly; although if we consider each of the two classes of alkalis as a whole, it is the least dissociated class which penetrates most rapidly. $N(C_2H_5)_4OH$ affords excellent confirmation of the rule. The substitution of four C_2H_5 groups for four H atoms gives a substance whose degree of dissociation, unlike the lower amines, is comparable with that of the strong inorganic hydroxids, Na or Ba, and correspondingly its power of penetrating cells is likewise limited and comparable with that of Na or Ba.

According to Overton, ammonia and the primary, secondary and tertiary amines are soluble in lipoids, while tetramethyl ammonium hydroxid is not. There is, then, a relation between lipid solu-

³ A different strain of *Paramæcia* was used in testing this alkali and it is possibly out of place in the above series.

bility and penetrating power but it does not follow from this that the cell surface is lipoid in nature, an hypothesis which is at present far from proved.

In conclusion I wish to emphasize again the very marked difference between the two classes of alkalies, strong and weak, in power of penetration. I have recently investigated and confirmed the relation for the cells and tissues of a large number of marine organisms. The two groups differ most markedly in physical properties with respect to degree of dissociation, lipoid solubility, and probably also in their power of lowering the surface tension of water. It is only by taking into account such marked differences as those above mentioned that any theory of permeability and of penetration can be built up.

ON THE ABSORPTION AND DISTRIBUTION OF ALUMINIUM FROM ALUMINIZED FOOD

MAX KAHN

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York)

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I. INTRODUCTION

In a paper entitled "Some objections to the use of alum baking powder," Professor Gies¹ recently called attention to the significant results which he and his collaborators have obtained in studies of the effects of aluminium compounds.

At the beginning of the paper he summarized the general findings in the following terms: "These studies have convinced me that the use in food of alum or any other aluminium compound is a dangerous practice. That the aluminium ion is very toxic is well known. That 'aluminized' food yields soluble aluminium compounds to gastric juice (and stomach contents) has been demonstrated. That such soluble aluminium is absorbed in some degree and carried to all parts of the body by the blood can no longer be doubted. That the organism can 'tolerate' such treatment without suffering harmful consequences has not been shown."

At the end of the paper Professor Gies says: "That absorbed aluminium may be distributed to all of the tissues and that such aluminium may *accumulate* there, to some extent, is a probability which has been considered but which has not yet been adequately

¹Gies: Journal of the American Medical Association, 1911, lvii, p. 816.

investigated." The practical importance of such a study is so evident that comment is superfluous. At Prof. Gies' suggestion the experiments described below were conducted for the purpose of extending our knowledge in this particular direction.

II. DESCRIPTION OF THE EXPERIMENTS

Preliminary supplies and conditions. The experiments were conducted on normal, healthy dogs, kept in cages which have long been in constant use in this laboratory.² The animals were fed daily at 9 A. M. The diet consisted, with the exceptions hereinafter noted, of 10 grams of hashed lean beef (taken from large supplies preserved in a frozen condition),³ 15 grams of biscuit baked by the process detailed below, 3 grams of lard, 1 gram of bone ash and 35 c.c. of water—*per kilo of weight of the dog*. The baking of the biscuits was done according to the directions of Professor Mallet.⁴ The flour selected was of the best quality for family use. Three large bags of Hecker's flour were bought. Each bag was emptied into a large porcelain dish and the flour thoroughly mixed. The flour from each bag was kept in large, wide mouthed, glass stoppered bottles. A sample of flour from each bag was analyzed for aluminium, but not a trace of aluminium could be found in the portions examined. In Prof. Mallet's experiments, "the flour used yielded in the ash the equivalent of less than 1 mg. of Al_2O_3 for the largest quantity of bread eaten by any of the subjects."⁵

"Bob White" baking powder was used. This is a "straight alum" powder. It was purchased in the open market. The label on each package gave the following summary of the contents: *Corn starch, sodium aluminium sulfate* (32.75 per cent.), *sodium bicarbonate*.

The ingredients for the baking were mixed in the following proportions: Flour, 550 grams; common salt, 8 grams; sucrose, 5 grams; "Bob White" baking powder, 16 grams; water, enough to make a biscuit dough. Before the water was added, the flour, baking powder, etc., were thoroughly mixed, to insure uniform

² Gies: American Journal of Physiology, 1905, xv, p. 403.

³ Gies: *Ibid.*, 1901, v, p. 235.

⁴ Gies: Journal of the American Medical Association, 1911, lvii, p. 817.

⁵ Gies: *Ibid.* (footnote).

distribution of all the ingredients. The dough was well kneaded and baked in tin pans very thoroughly (more so than the housewife's ordinary biscuits). The biscuits were very crisp and toasty when removed from the oven.

I analyzed several 20 gram portions (A—D) of the baked biscuit from several days' baking, with the following results for aluminium in terms of Al_2O_3 :

A—3.36 mg.; B—3.61 mg.; C—2.28 mg.; D—4.16 mg.

Method for the quantitative determination of aluminium. The results of Dr. Steel's work in this laboratory, and my own experience with the process, led me to adopt the following method of analysis, as recommended by the Association of Official Agricultural Chemists.⁶

Obtain an aliquot portion of the available acid solution (after oxidation, *vide infra*) and remove any contained silica. Mix the liquid with sodium phosphate solution in excess of what is required to form normal aluminium phosphate. Add sufficient ammonium hydroxide solution to effect complete precipitation of the aluminium phosphate after thorough stirring. Then add hydrochloric acid solution, drop by drop, until the precipitate completely dissolves. Heat the liquid to about 50°C . and mix with it, at that temperature, a considerable excess of 50 per cent. ammonium acetate solution and also 4 c.c. of 80 per cent. acetic acid solution. As soon as the precipitate of aluminium phosphate (mixed with iron phosphate) has sedimented, collect it on an ashless filter, wash it with hot water, ignite it and then weigh the residue.

In an aliquot portion of the original acid liquid determine the amount of iron by the Zimmerman-Reinhardt method.⁷ The calculated amount of FePO_4 is then subtracted from the weight of the mixed AlPO_4 and FePO_4 .

Many determinations were made by this method in mixtures of pure standard solutions of ferric chlorid and aluminium sulfate, and perfectly accurate results were obtained.

Preliminary oxidation of the analyzed tissues. Every tis-

⁶U. S. Dept. of Agriculture, Bureau of Chemistry, Bulletin 107, 1908.

⁷Mixer and Dubois: Journal of the American Chemical Society, 1895, xvii, p. 405.

sue analyzed by the method described above was first subjected to oxidation.⁸ The weighed tissue was placed in a Kjeldahl flask and concentrated nitric acid solution added to it. The flask was heated slowly at first and then more vigorously until the solution became clear, when a moderate excess of nitric acid solution was added and the liquid boiled down to a small volume. The fluid was then treated with a fairly large volume of concentrated sulfuric acid solution for the expulsion of the nitric acid and the complete oxidation of any residual organic matter. The sulfuric acid mixture was boiled for at least two hours after it became colorless, in order completely to eliminate NO_2 . The residue was then dissolved in water, made up to volume, and the iron and aluminium determined as described above.

Condition of the animals throughout the experiments. The experiments were conducted on four full grown healthy dogs. The feedings of aluminized bread were begun on June 29, 1911, and in each experiment were continued daily for about nine or ten weeks until the day on which the dog was killed. All of the animals appeared to thrive. They were weighed once a week. All of them gained weight during the long periods of confinement in the cages. The urine of each dog was collected every twenty four hours and was measured. Aluminization of the food appeared to be without effect on the daily average output of urine.

Operative procedure at the end of each of the main experiments. The dog, in each case, was bled to death from the femoral or iliac arteries. No general anesthetic was employed. Cocain was injected in every case over the site of the vessel to be exposed. None of the dogs showed any signs of pain during the operations.

Tissues selected for analysis. The tissues or organs named below were taken immediately after the death of the animal and at once placed in large, thoroughly cleansed, wide mouthed glass stoppered bottles: Bile (and gall bladder), blood, bone (femur and a piece of flat-bone from the skull), brain, heart, kidneys, liver, muscle from the thigh, pancreas and spleen. Before removing the gall bladder or the kidneys, they were tied off, so that no bile or blood

⁸ Steel: American Journal of Physiology, 1911, xxviii, p. 97.

could ooze out and vitiate the analytic results. The liver was next to the last organ to be taken out. The heart was the last to be removed. In each case the heart was cut open and quickly flushed with distilled water. Before the body was opened, and while the parts were being removed, special care was taken to prevent accidental introduction of foreign matter.

III. FIRST EXPERIMENT: DURATION, TWO MONTHS

The first experiment was started on June 29, 1911. The dog (male) weighed 8.66 kilos. He was given the following mixed diet: Meat,⁹ 85 grams; aluminized biscuit, 128 grams; lard, 25.5 grams; bone ash, 8.5 grams; water, 298 c.c. The dog gained weight on this diet.

On (and after) July 27, when the animal weighed 9.65 kilos, the lard was omitted and the same weight of meat substituted.

On August 29, when the dog weighed 10.72 kilos, and the experiment had been in progress for two months, the animal was killed.

The dog was bled to death from the left femoral artery under cocain anesthesia. The blood was defibrinated as it accumulated. About 510 grams of blood were removed. No more could be drawn from this artery. The heart beat was strong, and the respiration deep and vigorous. The animal seemed to stand well the loss of blood and gave no evidence of distress. The dog was allowed to lie on the board for one half hour. Cocain was then injected over the region of the right anterior iliac artery, and 155 grams more of blood were withdrawn. The blood clotted very slowly. The bleeding was very thorough. No free blood was noticed in any of the removed organs except the heart. In the heart the quantity of residual blood was very slight.

The total amount of blood removed was 665 grams. Of this quantity, 210 grams were oxidized, made up to 500 c.c. and 50 c.c. portions of the liquid analyzed for aluminium. The total volume of blood (655 grams) contained the aluminium in 10.9 mg. of Al_2O_3 .

The results of the analysis of the tissues for aluminium are summarized in Table 1.

⁹ Several samples of meat were analyzed for aluminium. None was found.

TABLE I.

Amounts of Al_2O_3 obtained from the tissues of the dog in the first experiment

Tissue	Weight Grams	Total mg.	Amount of Al_2O_3 Per 100 Grams mg.
Bile	110	14.1	13.0
Blood	655	10.9	1.7
Bone (femur)	48	0.8	1.6
Bone (skull)	62	None	—
Brain	68	None	—
Heart	75	None	—
Kidneys	70	4.2	6.0
Liver	179	6.4	3.6
Muscle	100	3.2	3.2
Pancreas	39	1.9	5.0
Spleen	21	1.7	8.0

In order to get rid of precipitated calcium sulfate in the analysis of bone, the clear supernatant fluid was decanted after the addition of sulfuric acid. In this process slight loss of aluminium may have occurred.

IV. SECOND EXPERIMENT: DURATION, 55 DAYS

A small female dog, weighing 5.68 kilos on June 29, was given the following daily diet: Meat, 57 grams; aluminized biscuit, 85.5 grams; lard, 17.0 grams; bone ash, 5.5 grams; water, 200 c.c.

On (and after) July 27, when the dog weighed 6.45 kilos, lard was omitted from the food but the same weight of meat was substituted.

On August 23, 55 days after the beginning of the experiment, the dog was killed. It weighed 6.64 kilos.

The dog was bled to death from both femoral arteries simultaneously under local cocain anesthesia. The dog was almost perfectly exsanguinated. Upon opening the abdomen the tissues were seen to be very pale, and no points of bleeding were noticed. The organs enumerated in Table 2 were removed. The liver was next to the last to be taken out. A very slight contamination of the liver with bile was unavoidable in this case.¹⁰ The heart was the last organ to be removed from the body.

¹⁰ Very great care was exercised in the removal of organs. Rubber gloves were used and after each organ was removed, the gloved hands were washed in a running stream of distilled water. The same precaution was taken with the instruments.

The analytic results are given in Table 2.

TABLE 2.

Amount of Al_2O_3 obtained from the tissues of the dog in the second experiment

Tissue	Weight Grams	Amount of Al_2O_3	
		Total mg.	Per 100 Grams mg.
Bile.	73	2.0	2.7
Blood	398	4.5	1.1
Bone (skull)	57	None	—
Brain	28	—	—
Heart	32	None	—
Kidneys	42	0.5	1.2
Liver	89	4.9	5.5
Muscle	100	2.8	2.8
Pancreas	18	3.7	20.5
Spleen	19	1.2	6.3

V. THIRD EXPERIMENT: DURATION, 52 DAYS

A female dog, which weighed 9.34 kilos on July 17, was used in this experiment. It was fed daily, until Sept. 7, on the following diet: Meat, 94 grams; aluminized biscuit, 143 grams; lard, 30 grams; bone ash, 10 grams; water, 350 c.c. On Sept. 7, when it was killed, the dog weighed 10.5 kilos.

The dog was bled to death from both femoral arteries simultaneously under local cocain anesthesia. About 620 grams of blood were drawn off. It was noticed that the dog had an irregular heart beat, that the arteries were very flabby and that the blood clotted very rapidly. The organs were removed in the usual order and with the customary precautions. The peritoneum showed many adhesions. The kidney on the left side was normal in position but larger in size than the kidney on the right side. The right kidney was smaller than normal, calcareous in part and placed horizontally under the diaphragm, with the pelvis pointing down. Where the kidney should have been on the right side, there was a small cyst containing black fluid. The genitals showed no anomalies. The pancreas was very small.

The bile and gall bladder were tied off as usual, but some bile escaped and contaminated a portion of liver which was quickly cut off. This portion weighed 53 grams and was not analyzed. The rest of the liver was altogether free from the bile which had escaped from the bladder. The analytic results are presented in Table 3.

TABLE 3.

Amount of Al_2O_3 obtained from the tissues of the dog in the third experiment

Tissue	Weight Grams	Amount of Al_2O_3	
		Total mg.	Per 100 Grams mg.
Bile.	32	6.7	20.9
Blood	620	10.7	1.7
Bone (femur)	41	1.1	2.7
Brain	75	None	—
Heart	97	0.1	0.1
Kidneys	60	1.5	2.5
Liver	288	5.3	1.8
Muscle	100	1.7	1.7
Pancreas	23	1.0	4.3
Spleen	18	2.0	11.1

From the tabulated data of these experiments, several deductions become self evident. Aluminium did not accumulate in the blood, but some of the tissues showed marked tendencies to accumulation. Aluminium seems to be abundantly excreted in the bile, as the results of the first and second experiments show with special emphasis.

That the aluminium in these tissues cannot be regarded as arising from residual blood is evident from the analytic data. The animals were very thoroughly exsanguinated, and the proportion of residual blood in any tissue was negligible.

TABLE 4.

A summary of the data in tables 1-3

Tissue	Dog I.			Dog II.			Dog III.		
	Weight Grams	Total mg.	Al_2O_3 Per 100 Gm. mg.	Weight Grams	Total mg.	Al_2O_3 Per 100 Gm. mg.	Weight Grams	Total mg.	Al_2O_3 Per 100 Gm. mg.
Bile	110	14.1	13.0	73	2.0	2.7	32	6.7	20.9
Blood	665	10.9	1.7	398	4.5	1.1	620	10.7	1.7
Bone (femur)	48	0.8	1.6	—	—	—	41	1.1	2.7
Bone (skull).	62	None	—	57	None	—	—	—	—
Brain	68	None	—	28	—	—	75	None	—
Heart	75	None	—	32	None	—	97	0.1	0.1
Kidneys	70	4.2	6.0	42	0.5	1.2	60	1.5	2.5
Liver	179	6.4	3.6	89	4.9	5.5	288	5.3	1.8
Muscle	100	3.2	3.2	100	2.8	2.8	100	1.7	1.7
Pancreas	39	1.9	5.0	18	3.7	20.5	23	1.0	4.3
Spleen	21	1.7	8.0	19	1.2	6.3	18	2.0	11.1

VI. ELIMINATION OF ALUMINIUM IN THE URINE

Although it was not the purpose of these experiments to determine the quantitative excretion of aluminium in the urine, we concluded, nevertheless, to collect some urine and analyze it for aluminium.

Special precautions were taken in the collection of this urine. A fourth dog, a female weighing 13.85 kilos on June 29, was fed daily thereafter for nearly three months on the following diet: Meat, 140 grams; aluminized biscuit, 210 grams; lard, 42 grams; bone ash, 14 grams; water, 490 c.c.

The dog gained steadily in weight. On September 13, the special urine collections were started. It was obvious that under the dietary conditions of these experiments we could not collect urine in the receiver under the cage, because in this way the urine would surely be contaminated by feces containing aluminium. Catheterization was avoided for fear of setting up a cystitis. Prof. Gies suggested the expedient of occasionally taking the dog out of the cage, allowing her under control to walk about the laboratory, and collecting urine in a clean porcelain dish as it was passed. In this way 610 c.c. of uncontaminated urine were collected during a period of about ten days (Sept. 13-23). All other fractions of the urine were rejected.

The uncontaminated urine was divided into two equal portions. Each portion was evaporated to a small bulk, then oxidized with nitric acid and sulfuric acid, and the aluminium determined, with the following results: Al_2O_3 in part **A**, 6.2 mg; Al_2O_3 in part **B**, 5.5 mg.; average amount of Al_2O_3 per 100 c.c., 1.8 mg.

VII. GENERAL CONCLUSIONS

1. When biscuits baked with alum baking powder are fed in a mixed diet to dogs, aluminium passes in considerable amounts into the blood.

2. Such absorbed aluminium circulates freely and, although it does not show a tendency to increase proportionately in the blood, it accumulates to some extent in various parts of the body. The bile contains a particularly large amount of aluminium under such circumstances. The pancreas, spleen, liver, muscle and kidneys con-

tain considerable amounts, while the brain and heart seem to resist accumulation of aluminium. The long bones, under the conditions of these experiments, contained aluminium. The flat bone of the skull did not contain aluminium.

3. Aluminium, when ingested in aluminized food under the conditions of these experiments, is absorbed in part and is excreted, to some extent, in both the bile and urine.

I wish to express my sincere gratitude to Prof. Gies for his kind suggestions and material aid throughout this work.

A RETROSPECT IN BIOCHEMISTRY

CHARLES A. DOREMUS

To the University of Pennsylvania belongs the honor of founding, in 1765, the first medical school in this country, and to Dr. Benjamin Rush, the eminent physician, that of being the first American professor of chemistry. He was succeeded by Dr. James Hutchinson and, on his death, in 1794, Dr. Rush offered the chair to Joseph Priestley, who had emigrated to this country, landing in New York on June 4 of that year, and who had taken up his residence in Northumberland, Pa. Priestley, after due consideration declined the honor, largely because it would have necessitated his being away from his family in the winter.

The discoverer of "vital air" or oxygen; of the power of the plant to so act on the carbon dioxide of the expired air under the influence of sunlight, as to render the air again respirable—"to mend the air" as Dr. Franklin put it—is thus closely linked with the beginnings of biochemistry in this country.

The Chemical Society of Philadelphia was founded in 1792, and Dr. Priestley was deeply interested in its welfare.

It was before this Society that in 1801, Dr. Robert Hare, then but twenty, read his memoir on the oxy-hydrogen blow-pipe. He showed what intense heat, sufficient to fuse platinum and to render the oxides of the metals of the earths brilliantly incandescent, could be obtained through the union of these gases. He subsequently became the professor of chemistry at the medical school of the University of Pennsylvania.

We are indebted to Priestley for the isolation not only of oxygen, but also of nitrous oxide, ammonia, hydrochloric acid, sulphur dioxide, and silicon fluoride while in England, and for the discovery of carbon monoxide while at Northumberland, Pa. He also investigated nitric oxide, and carbon dioxide, the latter in relation to respiration and combustion. The interdependency of animal and plant life was established through his researches.

Thus biochemistry and chemistry itself were intimately connected with the instruction given in medical schools throughout the original States of the Union, and as the tide swept westward this condition persisted until the end of the nineteenth century.

Professor John Torrey, whose name is so closely linked with botany, was professor of chemistry at the College of Physicians and Surgeons in New York, and Professor Benjamin Silliman, distinguished in so many branches of science, was Professor at Yale College, as it was then known.

When the medical department of the New York University was founded in 1841, Professor John W. Draper was appointed professor of chemistry, and as he was deeply interested in biochemistry, as shown by the title of his most important work, "The Forces which Produce the Organization of Plants," N. Y. 1844, and was the author of a text book on physiology, his teaching elucidated its fundamental principles. His elaborate researches on light, the spectrum, photography, the tithonic, or dark rays, osmosis, and many other chapters of the border land between chemistry and physics, are some of the most notable contributions ever made to science. When the American Chemical Society was founded, in 1876, he was elected president.

But while chemistry engaged the attention of all students of medicine and was given more fully in medical schools than in the colleges, it was taught in lectures, and the students had no opportunity to perform experiments. The wonderful influence of Liebig had not yet even spread through Germany, but was rapidly gaining favor at the end of the forties. Not until 1850, when the New York Medical College was founded, were medical students offered the advantages of laboratory instruction, but required to take the course quite like their dissecting in connection with the study of anatomy. This innovation was secured through the zeal of Professor R. Ogden Doremus, to whom the chair of chemistry was confided. A pupil of Draper, and his assistant for many years, he had, through a visit to Europe in 1848, become imbued with the new doctrine of laboratory instruction and was determined to initiate it in the new institution. He was also professor of chemistry in the N. Y. College of Pharmacy. The students of that institution, then

homeless, were given evening instruction in the lecture room and laboratory of the N. Y. Medical College, from 1850 to 1860.

The requirements for entrance to, and graduation from, a medical school were then and until about fifteen years ago simpler than at present. Neither high-schools nor colleges had the facilities for instruction in science which they now possess. Indeed most of our universities were then but colleges, and the antagonism of many religiously minded people to the teachings of science was strong. The medical student selected a preceptor, with whose practice he became somewhat familiar. Later the medical colleges acted as preceptors. The course of study involved attendance at two courses of lectures and clinics, and the student heard in his second year substantially the same lectures he had heard in the first. He had clinical privileges the second year not accorded the first. It was possible to get through the two courses (one during the winter, the other during the summer) in the same year, by going to two different institutions whose terms were so related. The title of a paper read before the Medical Society of Philadelphia, in 1800, by John Redmond Coxe, M.D., "A Short View of the Importance and Respectability of the Science of Medicine," indicates the conditions attending the founding of the early schools.

The innovation of a three-years course when first attempted by Bellevue Hospital Medical College, was objected to by the other medical schools, and the present system of an examination at the end of each of the four years course is of recent date. The prerequisite of a college course is not yet exacted by all schools of medicine.

The College of Physicians and Surgeons had no adequate instruction in either physiology or pathology for the first forty years of its existence, and it was not alone in this respect. Indeed, the study of anatomy was hampered by the fact that dissecting material was not easily had, and the Act which legalized the obtaining of such, was passed under the title of "An Act to prevent the Desecration of Graveyards."

The introduction of experimental demonstrations of vital processes by experiments on animals, which at one time was conducted before the classes, was later almost abandoned though now

such demonstrations are carried out somewhat differently in connection with bacteriology and experimental medicine and surgery.

The course in chemistry followed in the medical schools until the last decade of the nineteenth century was largely one on general chemistry, with special emphasis on the portions relating to life processes, to sanitation, water and foods, and toxicology while the discussion of drugs and pharmaceutical preparations was delegated to the chair of therapeutics. There was small chance, therefore, for the systematic study of biochemistry as now understood. Laboratory instruction consisted of a study of the qualitative and quantitative tests for the more important inorganic and organic substances having a relation to medicine, followed by work on the urine, milk, blood and other animal fluids and tissues. The foregoing, with experiments on animals, and the action of poisons, constituted the routine work of the students at the New York Medical College.

At that period there was great interest in obtaining electric phenomena from electric fish, from various tissues and in studying the effects of the current on the animal organism. Matteucci constructed a battery from the demi-thighs of frogs, a silver wire attached to the exterior of the denuded muscle being the positive, another to the interior of the thigh muscle the negative, electrode. If these were connected to a galvanometer, the deflection of the needle demonstrated the presence of a current. The slaughter houses in New York City were located North and East of 23d Street and Lexington Avenue. It was possible to get a freshly slaughtered bullock's head and have it cut off below the larynx. When quickly taken to the College on East 13th Street, the tissues were still sensitive enough to show the effect of the voltaic current. With one pole of a battery thrust into the spinal cord, whenever the other was touched to the outer surface of the head, the facial muscles could be made to contract, the nostrils to dilate, the eyes to roll, the tongue protrude, and sometimes a semi-bellow could be caused by making the wind-pipe suddenly contract below the vocal cords.

Aldini constructed a bovine battery of bullock's heads like the frog battery alluded to above. The rythmic pulsations of an extirpated turtle's heart are easily shown on a screen, as also the circulation of the blood in the omentum of a pithed frog. Such experiments given in a lecture make a never to be forgotten impression.

In 1859 the Long Island College Hospital was founded, and there, in 1861, Professor Doremus introduced laboratory instruction in chemistry. The other New York Colleges established their laboratories much later. The writer organized that of the Medical Department of the University of Buffalo in 1879, and that of the American Veterinary College in New York in 1892.

While the physician is interested in the action of drugs as remedial agents, the toxicologist has to consider them also from the standpoint of criminology. The art of poisoning is, alas, ancient and it often requires special skill to detect the cause of death in cases of suspected homicide. Investigations of this kind have to conform to the legal procedure of the land in which the case is tried. We have trial by jury, and oftentimes the antiquated coroner's office (no longer extant in Massachusetts) is the first court before which the inquiry is made. Under our system no defendant will consent to having a chemist representing him witness the analysis, and attest its findings as correct. The analyst for the State must present the result of his investigation in plain and unbiased language to the jury, and must submit to a rigid cross-examination at the hands of defendant's counsel. The evidence he has secured must be convincing, and inspire the confidence not only of the jury but also of the community. When in 1858-9 an important case of suspected murder by poison was brought to the attention of the authorities, the district attorney requested Professor Doremus to make the investigation. This he consented to do if radical changes were made in the methods previously followed: A special laboratory to be provided, blank tests to show that all chemicals and porcelain, glass, or other vessels were free of taint, and other precautions, unnecessary to dilate on here, to be taken. These requests were acceded to and the body of the woman, which had been buried a year, was exhumed and the autopsy and analysis conducted in the improved manner. On the finding of arsenic in the viscera, and various tissues of the body, the soil surrounding the grave, the coffin, its appurtenances, and other objects were analyzed with negative results.

To refute the contention of the defense that arsenic might be found in any human body, the entire cadaver of a woman who had

been frozen to death, was analyzed and no arsenic found. Probably this was the first, if not the only time, that an entire human body had been subjected to chemical analysis.

The prisoner was found guilty of poisoning his wife, and was executed. No other case of homicide by poison was tried in New York for a period of thirty years, though thereafter there were several other *causes célèbres* within a decade.

Experimentation in physiology in the fifties and sixties, though often brilliant and showing rare technique on the part of the teachers, was comparatively simple. Few colleges possessed any of the recently devised apparatus for recording the action of the pulse, the contractions of a muscle, and none of the more elaborate apparatus used now in the demonstration of the functions of the special senses. The institutions abroad had often very little. When Helmholtz was called to Berlin he depleted the cases at Heidelberg, for most of the apparatus had been presented to him as a gift by the King of Bavaria.

Professor Kühne, who succeeded Helmholtz, was more devoted to the chemical side of physiology, and inaugurated new lines of work which soon attracted so many students that a special building was erected for him. It is with the keenest pleasure that the writer remembers the hours spent in listening to his able lectures, and recalls his genial presence in the laboratory. There were but few students in Kühne's laboratory at that particular time (1871-2), Professor Gamgee of Manchester being one of them. In the following semester the writer pursued courses with Professors Ludwig and Drechsel in Leipsic. There were many students and post-graduate students attending the lectures on physiology, but he was the only student in the laboratory for physiological chemistry. Professor Drechsel was a splendid teacher and, devoted to research, we have him to thank for the elucidation of many problems.

Such were the conditions at two of the foremost German universities in 1871-2. The physiological building at Leipsic was commodious and Professor Ludwig had a two-hour period for his lectures, which enabled him to perform many experiments otherwise impossible. Many times animals were shown in an insensible condition, under artificial respiration, the preparation of the

specimen being conducted by assistants in the laboratory and the demonstration table then wheeled into the lecture room. This had great advantages, the chief one being that of the lecturer retaining the attention of his audience.

The *Jahresbericht für Thier-Chemie*, under the editorship of Richard Maly, appeared in 1871, a modest volume of 341 pages. Immediately after the Franco-Prussian war, the University of Strassburg was founded and Felix Hoppe-Seyler was installed as professor of physiological chemistry. In 1877 he began the publication of the *Zeitschrift für physiologische Chemie*.

It took some years to erect the splendid permanent buildings which make the University of Strassburg one of the finest in Europe, and when proper housing was effected the work of the Institute expanded. Pettenkoffer had built a respiration calorimeter in his laboratory in Munich; an improved one was constructed by Hoppe-Seyler. The study of the respiratory functions, and of the blood, of the absorption spectra and of the gases of the blood were some of the subjects of his investigations. Glass apparatus for mercury pumps could only be had in Europe at that date. Later, the advent of the Edison bulb revolutionized the glass-blowing industry on this side of the Atlantic.

Previous to 1880 our medical schools offered little or no opportunity for the study of physiological chemistry. When in 1877 Hoppe-Seyler wrote his excellent book on the subject, the writer secured his permission to translate it into English, and it would have appeared simultaneously with the German edition. No prominent publisher of medical books, either in New York or in Philadelphia could be found, however, who could see profitable sales, and the project had to be abandoned.

The influence of the French scientists must not be overlooked. The brilliant work of Claude Bernard, of Regnault and Reiset, of Robin, of Paul Bert together with many leaders in other departments of medical science, made Paris, up to 1870, the center of medical instruction abroad. But the German schools had been steadily forging ahead, and the writer was advised by Professor Wurtz, one of the most noted of French chemists, to repair to Germany, since the facilities for obtaining instruction in general

chemistry were far better there than in either France or England. There was really no advanced teaching of chemistry in this country at that time, most of the universities and technical schools doing splendid work for the science to-day had either not been founded or were just emerging from the college to the university stage. Immediately after the Franco-Prussian war, France revised her school system and outlined a progressive policy for the development of her higher institutions of learning. She had in Pasteur a master mind, whose wonderful researches created a new field of scientific endeavor and not only gave impetus to the study of biochemical problems, but also revolutionized the science of medicine, developed new industries and reconstructed old ones.

In looking over data referring to the decade of the seventies two important contributions are found to have been made in this country on respiration. Immediately after the isolation of oxygen and the recognition that animal life was maintained by it, it being, so to speak, like "a vestal virgin to the body, to keep alive the flame of life," there was a great misconception regarding the inhalation of pure oxygen. Arguing by analogy from the rekindling of a splinter of wood from a mere spark at its end when placed in oxygen, it was thought that similarly on inhaling oxygen one would "live too fast." In a prize essay on "Oxygen as a Remedy in Disease"¹ the results of experiments were described which showed that not only was this idea fallacious but that some of the experimenters abroad had fallen into an equally grave error, in supposing that oxygen was toxic because when an animal was put in an atmosphere of pure oxygen it died after a time. When the residual air was tested it was found that a splinter would rekindle from a spark. The presence of the carbon dioxide in this residual gas had been ignored. It was proved to be the poison, for when removed as formed, by placing caustic alkali in the oxygen along with the animal, the animal continued to live. Now, helmets are constructed which permit the removal of the carbon dioxide exhaled by the wearer, while the deficiency in volume is made up from a cylinder of compressed oxygen attached to the apparatus. Indeed if sodium

¹"Oxygen Gas as a Remedy in Disease," by Andrew H. Smith, M.D., *N. Y. Medical Journal*, 1870. Prize Essay of the Alumni Association of the College of Physicians and Surgeons, N. Y.

dioxide is used, the moist carbon dioxide from the lungs decomposes this compound, forming sodium carbonate, and releasing oxygen. Accidents to submarine boats are guarded against by the use of this compound.

The isolation of oxygen from the air, according to Tessié du Motay's process, was being conducted on a commercial scale. The 20,000 cubic foot holder supplied the gas to those who would take the trouble to visit the works and obtain permission to inhale the gas. Compressed in cylinders it was sent to the homes of patients and allowed to escape freely into the sick room. Great relief was found by those suffering from asthma, pneumonia, diphtheria, and other diseases, and unfortunately at no time since has oxygen been as easily at the command of the physician. It is to be hoped that the great demand felt in the commercial world for cheap oxygen will soon result in the development of some process to put it again at the command of all.

When the first Brooklyn Bridge was built a caisson was sunk on the New York side and on it the pier which carries the cables was erected. The work had to be done below the surface of the river and in compressed air. Never before had there been an opportunity to study so thoroughly the effects of compressed air on respiration. The gangs of workmen were put under scientific observation and control. Dr. Andrew H. Smith, after long study of the conditions and of the illness from which the men suffered, gave the name of "caisson disease"² to the serious, often fatal, character of the symptoms which developed after the men emerged from the compressed air. The rules he formulated have since been employed to protect the men while performing such dangerous tasks, now frequently occasioned by tunneling under rivers, or laying foundations.

When the highly improved respiration calorimeter was built at Middletown, Conn., and investigations with it were made by Atwater and his corps of assistants, the study of the metabolism of the human body at rest, at work of various kinds, and under the use of different foods, yielded a vast amount of information.

² "The Effects of High Atmospheric Pressure, Including Caisson Disease," by Andrew H. Smith, M.D., 1873. Prize Essay of the Alumni Association of the College of Physicians and Surgeons, N. Y.

In 1870 an elaborate research was made on the subject of the elimination of nitrogen under protracted muscular exercise. The long-distance walker Edward Payson Weston put himself under medical observation for five days prior, five days during and five days after a walk in which he covered three hundred and seventeen and a half miles out of the four hundred he expected to walk. An increase in the elimination was observed, results subsequently confirmed on the same individual abroad. He has, in his seventies, walked twice across the continent, from east to west and again from west to east.

In 1864 the Citizen's Association requested the medical profession to inform it of the sanitary condition of New York. The Report of the Council of Hygiene and Public Health was made in 1865. This was the first concerted action relating to such matters, and as a result the Health Department was subsequently organized and its work has grown to vast dimensions.

During the Civil War but two disinfectants were in common use—chloride of lime and potassium permanganate. Professor Alpheus B. Crosby, of the Bellevue Hospital staff, in a discourse "On a Lost Art in Surgery, Cleanliness," tells of the successful disinfection of the hospital by the use of three tons of chlorine gas generated in the wards, to abate serious infection from puerperal fever and septicemia. Present antiseptic methods were dawning.

There were attempts to regulate the purity of water supplies, and of food, but a long, very long campaign of education, the adjustment of interstate conditions and many other hindrances had to be overcome before even the present laws could be enacted and enforced.

No attempt has been made to more than note the phenomenal changes which took place in our educational system about 1880, when things educational shot up, as children sometimes take to growing. Inadvertently perhaps, someone administered a dose of extract of the pituitary body. At any rate the high schools, colleges, and universities became capable of preparing the student of medicine in chemistry so that the new courses in bacteriology and pathology could be better understood.

The field of view has not been extended to take in the whole

country nor to pick out the special instances where enthusiastic pioneers have labored so devotedly and so successfully. Their influence is not forgotten though unmentioned.

We must not expect a student to absorb and assimilate the mass of information which has been accumulating, nor must we expect him to submit to the cramming system as if he were a Strassburg goose with a fatty liver. We consider the problem of prolonging life to a hundred years as eminently biochemical. Why should we not, perhaps either by some of the new synthetic carbohydrates or new protein foods, adjust the seven ages of man, so that the present unduly extended educational period shall not be so disproportioned to his physiological development.

BIOCHEMISTRY

Its Place in the Curriculum of the College of Liberal Arts for Women¹

MARY LOUISE FOSTER

Instructor in Biochemistry, Smith College

It is with pleasure that I come here to plead for a place for biochemistry in the college of liberal arts for women. I am in hearty sympathy with the recent action of the Association of Collegiate Alumnae in urging the introduction of Home Economics into our colleges for women. In 1883, Mrs. Richards was the prime mover of the Sanitary Science Club, a group of women who felt the need of study of this kind and who declared in the introduction to the book which they published later that the "expenditure of time and effort had been amply repaid by positive and satisfactory results." Mrs. Richards was my most honored teacher at the Massachusetts Institute of Technology and later I was associated with her on a committee from the Women's Education Association appointed to devise a working program for a manual training school for girls in Boston. Our object was to make women efficient in their own domain, efficient at home, and we believed that, with the new knowledge which research had made available, training was necessary if this knowledge was to be diffused. It is instruction along these lines which our alumnae are now urging upon the trustees and faculties of our colleges. Classically educated myself, I got my first stimulus from Mrs. Richards for the work which has been a continuous happiness to me.

Dr. Eliot in an address before the Association of Collegiate Alumnae in 1908, said that the effort in the early years of the existence of women's colleges was to demonstrate the fitness, the mental capacity of women for the education which men had been

¹ Read before the American Home Economics Association in annual session at Washington, December 28, 1911.

receiving for years. Mary Lyon, busy in 1838 in founding Mt. Holyoke, demanded a higher education for women who might thus fit themselves to fill the places which were waiting for them. Mary Lyon was far ahead of her time. She foresaw that women would enter the teaching profession and knew that they needed preparation. What woman without special training would today dare to offer herself as a candidate for a school position? The Girls' Latin School founded in Boston in 1878 through the efforts of many women in that city, was an expression of the conviction of these women that, given an even chance, girls could prove themselves equal to the excessive demands of a classical education. The steady growth of this school with its rigidly maintained high standard has justified the belief of these founders. Wherever we turn, we find similar results: fears that college women would not marry are not borne out by statistics; forebodings that health would be ruined are removed by records of our gymnastic departments which show improved physical conditions, due probably to the regularity of college life; doubts of the ability of women to maintain lines of thought are dispelled by the long lists of women filling positions inside our colleges and outside, where intellectual worth and executive power are the prerequisites. Think for a moment of Miss Thomas at Bryn Mawr, of Miss Pendleton at Wellesley, and of Miss Woolley at Mt. Holyoke, to mention only a few.

The demonstration of our ability to cope with an education is complete. Now we face a new situation: what education is best? To quote Dr. Eliot again from the same address: "We are free now to arrange for an education for women which is specially adapted to the needs of women." And again: "The prime motive of the higher education of women should be recognized as the development in women of the capacities and powers which fit them to make family life more intelligent, more enjoyable, happier, more productive—productive in every sense, physically, mentally and spiritually." Time was when Dr. Eliot did not believe in higher education for women. I once heard of an episode which took place in a Harvard faculty meeting. President Eliot had been pleading the cause of Radcliffe, then known as "The Annex." One of the professors remarked that he remembered a similar meeting held in that very

room about ten years before, when the Chair had expressed an opinion, the very opposite to the present one. "Well, Sir," said Dr. Eliot, "if you are interested in that as a matter of history, I am very glad. As for myself, I hope I have learned something in ten years. We will proceed to vote," etc. He has ever since that time been our strongest advocate and we desire to realize his faith in us. And what does study of the present condition of college women show us? We find the classics and mathematics deserted after the requirement of the first college years has been passed, except by those who have marked talent for these studies or who wish to fit themselves to teach these subjects specifically. We find them electing enthusiastically courses in history, sociology, economics and literature—cultural courses all, and tending to fit the students to fill the places in the social community to which they must go on leaving college. We find alumnæ engaging in sociological work to such an extent that this is becoming a profession rivalling that of teaching, as eminently fit and proper for the educated woman.

What place does science fill? In my own college, Smith, a year's work in either chemistry or physics is required of every student; the class usually divides itself about evenly between the two subjects. Thereafter all work in these departments is elective. This year about twenty-five per cent. of the class in elementary chemistry have continued and twenty per cent. will continue through the rest of their college course. This is ten per cent. of the whole class. I suppose similar proportions prevail in other colleges. My study of the catalogs of our Eastern colleges divides them into two classes: those which teach and those which do not teach, home economics. The former, Simmons and Teachers' College, might be called technical schools; the latter, colleges of liberal arts. These technical schools are doing a splendid work, not only in training women for a professional career, but in raising the standard of living and in applying to daily tasks intelligent methods, lifting the drudgery of house-keeping into the scientific methods of the laboratory. The agricultural schools are offering courses to the farmers' wives. At a reception in Geneseo a few years ago during a Grange meeting held by the Cornell School of Agriculture, a woman talked enthusi-

astically about Mrs. Van Rensalaer's lecture and said, "I find I have been feeding my family on the things I ought not to have fed them on. I thought I was giving them of the best, whereas it was the worst possible combination. I am going to change." Perhaps she was reforming on insufficient knowledge but surely the desire that her family benefit by the improved methods was praiseworthy.

Cooking and sanitary science are being introduced into the grammar and preparatory schools all over the country. In the meantime what are our colleges of liberal arts for women doing to give their students a share in this widespread desire for better living conditions? Almost without exception they offer a single course in food analysis, of three or five hours in a single semester! This course was introduced as a concession to the demands of *alumnæ*, is taken only by the most advanced students in chemistry and is of an exceedingly technical character. What about the large numbers who graduate without taking more than the required chemistry? What of those who have taken no chemistry at all? Many of these begin at once to teach. They have not increased their knowledge of the benefits of science to the welfare of man. Is it not possible to introduce courses of less technical character? Statistics show that there are some 75,000 women in the colleges of the country. They should be the leaders, trained in logical thinking with power of concentrating their attention on the matter in hand. Could we infect them with our enthusiasm for scientific housekeeping, there would be no household problems!

Professor Giddings defines the *home* as the place of development of the social individual, economic and cultural. We have misplaced the emphasis hitherto; we are devoting our energies to the city and state as the place of development of the individual rather than to the home. There everything has long been done for his *cultural* development and now measures are being urged for his economic development. The economic valuation of a man is \$20,000. This is based upon efficiency, which is largely a matter of education and health. These then are assets for which we are all eager. The value of the former we have long recognized and have provided for in our school systems; the importance of the latter, health, is now coming to our attention. Modern methods are preventive rather

than curative. We have discovered a new truth in the old adage, "An ounce of prevention is worth a pound of cure." In olden times a surgeon washed his hands *after* an operation; now he washes them *before*! Insurance companies are providing nurses for their sick policy holders, a well man being of more value to them than a sick man. In the crowded city districts mothers are given instruction about the care of their babies and milk suited to the need of each child is provided at small cost. These precautions, education and proper diet, have decreased the infant mortality tremendously.

There is a carefully regulated diet for the invalid. But what about the well man? Does he not need the right food, food suited to his occupation, to maintain him in fit condition in order that he may keep for years at the point of maximum efficiency? Nature has given each of us a wide margin. Modern surgery removes organs and transplants organs and yet active life continues. We can best conserve life by conserving the energy which comes from the food we eat and the air we breathe. We have opened our windows and are less afraid of a draft than we are of breathing vitiated air, but we have not yet very generally given thought to the food we eat. As long as it is cooked according to familiar standards, we accept it. President Jesse said in 1905 that "women are the prime factors in society and should realize that life for themselves and others rests upon a physical basis and that life and health depend in large degree upon the choice of food, the preparation of it, household sanitation and household economics." We have not universally accepted this point of view. The attitude of the ordinary individual is well illustrated by a discussion between two students on the relation of chemistry and zoölogy. "Why," said one, "there is no relation. Zoölogy is life and chemistry is atoms!" We need to convince people that chemistry is life, that it is fundamental and that it enters into all the reactions of living. Panama has become a health resort through the agency of the scientist!

The chemistry of foods, the chemistry of life, animal and vegetable—biochemistry to use the new term—is very complex and it was not possible to teach it as a science until organic chemistry, pure and simple, had done its work of analysis and synthesis. But Pasteur, Fischer, Kossel, Ehrlich, to mention only a few, have

advanced our knowledge of the carbohydrates, the proteins, the pigments and the metabolic processes in the cell, until now we have the material at hand for scientific study of foods and food values in our college laboratories. Any pure food law is rendered useless unless we have intelligent buyers. So long as the housekeeper insists upon using white flour, the manufacturers will find means to bleach the flour. Scientific training makes us face facts and find solutions for the conditions which are inimical to our welfare.

Wm. H. Edwards, commissioner of street cleaning in New York City, is reported as saying to the Vassar students: "The housewife plays a more important part than she realizes in keeping the streets of our cities clean. Her standards of cleanliness in the home must be in evidence outside the house. Regularity in the output reduces congestion of refuse; proper separation of refuse adds to the sanitary conditions of removal. No woman who is a good housekeeper will throw papers or waste in the street, and most street litter was first thrown away by some thoughtless hand. Her husband will not throw his newspaper in the street. Her children will not litter our thoroughfares with all sorts of rubbish, but will take a certain pride in helping to keep the streets clean. The teachers in our public schools have the influencing of the children largely in their hands. Every teacher has the opportunity to teach patriotism to every little citizen by training him in loyal thought regarding his own city. She need go no further at first."

At Smith College in the department of chemistry we believe that scientific work is cultural; that scientific habits of thought are liberalizing; that it is possible to lay the foundations for a professional career whether that career be home-keeping or industrial chemistry. The course in general chemistry may be followed by a course of lectures on the applications of chemical facts and principles to common life. We have a course in sanitary chemistry, a very technical course, such as is given at the Massachusetts Institute of Technology; one which makes a student intelligent on the methods in use in our government and analytical food laboratories, and which studies the problems of public health. We have also a course running through the year in what we have called "Studies in Fermentation." This takes up the action of yeast and of various enzymes on the carbohydrates and the proteins, with special reference to the

products and by-products which are formed; it considers the action of bacteria on proteins with special reference to the chemical changes which they produce. I use the familiar yeast to show that the cell is the unit of life and that many metabolic processes take place there. The study of the carbohydrates and of the relation of the yeast cell to the carbohydrates takes us into the domain of biochemistry. Molds and bacteria follow naturally. After we have learned the technique of bacteriology we isolate and study the organisms gathered on a Petri dish during a few seconds' exposure in a room just swept or one just vacated by a class. We isolate and study the organisms collected from the daily supply of milk. Our constant object is to compare the standard uncontaminated material with the fermented or changed material. The importance of the by-product is dwelt upon and the relation of the reaction "in vitro" to the reaction "in vivo" is emphasized. Much of the reading in connection with the course has to be magazine reading and this, too, lays stress upon the fact that most of this work is recent, although the beginnings date far back, before there was any science of chemistry.

There has been one unexpected development of this course in which the faculty of the department are interested. Fifty per cent. of the students have entered laboratories as assistants or have gone to medical schools. There seems here an opening, rapidly enlarging field for women workers. The eagerness with which these opportunities are sought shows how ill adapted many of our alumnæ feel for teaching. It is no part of our intention to become distinctly a training school for technical chemists and yet our students want to know that the long afternoons spent in the laboratory do prepare them if necessary to do a real work with a paying wage. In our academic circles as elsewhere the stamp of success is that "it pays." There seems to be slowly developing a consciousness of the importance of this kind of work for women, and opportunities for its practice are rapidly increasing. It is an interesting development and as such we shall do what we can to make it prosper. It is not, however, our chief aim. Our chief aim is to give these students a liberal culture for the development of such trained minds that logical thinking and sound opinions can be brought to the solution of the problems which they meet.

The most absorbing work of our research laboratories today is bridging the gap between the chemistry of the test tube and the chemistry of the living cell, the most wonderful laboratory of all. But we know that the discovery today in the laboratory is the commonplace necessity of the world tomorrow. Already much of this biochemical metabolism is familiar to us and has revolutionized many industrial and agricultural processes. Why should we call on these women to go out without the faintest inkling of the existence of these discoveries? Why should they perpetuate the theory that alcohol and carbon dioxide are the sole products of the fermentation of sugar when the list of known by-products is already large and we are not yet at the end?

We are constantly hearing about the high cost of living. Mrs. Richards said it should be the "cost of high living." But whichever it is, we read about commissions of national and international scope appointed to investigate these matters and find a solution. Crops are large, we have world markets and yet prices continue to go up. Let us inform the women; they are the buyers, very largely, and as such affect the supply and demand. If they were resourceful and could substitute some other method in the household for the one which no longer pays, as is the way in the industrial world, this difficulty of high cost of living would disappear. It is not protection by government that we need so much as a wider education in the practical things of daily living. Pasteur gave his attention to the infinitely little and left the world his everlasting debtor. What is the use of spending years in the study of literature and art only to find ourselves at the end of it unable to cope with the industrial condition in our own household! Why try to regenerate the so-called slums all winter, only to be forced to live in a summer boarding house where dairy and culinary methods are the methods of our grandmothers? First reform our own homes, then reform others. Demand for more up-to-date methods, bacterial and chemical, would stimulate the supply and decrease the cost. But we cannot demand what we know nothing of. Let us educate the women in our colleges in the practical art of living, make them leaders, pioneers, missionaries if you will, for more rational conditions in all matters which pertain to living.

SUGGESTIONS TO TEACHERS OF BIOCHEMISTRY

2. Methods of applying the biuret test

JOHN L. KANTOR AND WILLIAM J. GIES

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York)

About three years ago the senior author was given the agreeable duty of planning the equipment of the laboratory of physiological chemistry in the newly erected Household Arts building at Teachers College. When the time came to decide upon the capacity, number and labeling of the reagent bottles to be purchased for each laboratory desk, it was found that we could not provide all the desired reagents (32), in uniform sets of containers in the available shelf space, if bottles of greater capacity than 4 oz. were selected for the purpose. We then proceeded to study the situation from its practical standpoints, especially with regard to the use of, and the need for, each of the intended reagents.

From the esthetic point of view, the selection of 4 oz. bottles left nothing to be desired. It seemed likely, however, that if such small bottles were employed, some of them would be emptied so frequently, and would have to be filled so often, that considerable loss of time would be involved in their use. On the other hand, that the smallness of the supply of each particular reagent would encourage economy and neatness in the use of all the reagents, was equally probable.

The only reagent which this study led us to fear would be employed so freely as to cause embarrassment because of frequent emptying and refilling of the bottles, was 10 per cent. sodium hydroxid solution (the preferred representative of the caustic alkalies). The reasons for this fear were the frequency of the need for this reagent by the student, both for general purposes and in applications of the biuret test. The usual tendency to excessive alkalization of needlessly large portions of the liquids under exam-

ination for protein in the biuret test emphasized this apprehension.

At that time we felt satisfied that Benedict's modification of the Fehling reagent was superior to the Fehling reagent itself for the detection of reducing carbohydrates, and accordingly a bottle, labeled FEHLING-BENEDICT REAGENT, was reserved for this liquid in the series of reagents referred to above.¹ In seeking a way out of the difficulty attending the use of excessive amounts of alkali in the biuret test (the difficulty to which we have just alluded), we thought of the possibility of using the Fehling-Benedict reagent itself. We found, however, that the Fehling-Benedict reagent would not yield satisfactory results as a substitute for copper sulfate and caustic alkali in the biuret test (the original Fehling solution being better for the purpose), and that the selected reagent for the detection of reducing carbohydrate would not serve the double purpose we had in mind.²

Fehling reagent, as is well known, may be used satisfactorily for the detection of protein when comparatively large proportions of protein are present in the material under examination, but when relatively small quantities of protein are contained, the addition of the Fehling reagent conveys an excess of copper into the medium and the typical color of the test may be wholly obscured or its recognition may be rendered uncertain. A reagent that would convey a small proportion of copper, with a relatively large amount of caustic alkali, was an alternative which naturally came to mind as these predicaments were considered.

Ultimately, seeking such a reagent, the senior author tried one of the simplest possible combinations. A few drops of 3 per cent. copper sulfate solution were added to about 100 c.c. of 10 per cent. NaOH solution. The precipitated cupric hydroxid was dissolved entirely when the mixture was shaken. The resultant liquid was distinctly and uniformly blue. Several such additions of copper sulfate solution were made to the blue liquid with the same result so far as solution of the precipitated cupric hydroxid was concerned, but the blue color was intensified. Finally, after thus imparting a

¹ Fehling solution was not included in the series.

² The alkalinity of the Fehling-Benedict reagent is too weak, to say nothing of the excess of copper in the liquid, from our standpoint—two reasons for its excellence as a reagent for the detection of reducing carbohydrate.

pronounced blue color to the alkaline solution, some of the liquid was added to a dilute solution of egg white. A beautiful biuret reaction was instantly obtained *with a single drop of the reagent in about 10 c.c. of the protein fluid.*

Dozens of similar tests immediately afterward, from many standpoints, made it very evident that the coppered caustic alkali solution would meet every ordinary expectation as a biuret reagent. It yielded sharper tinctorial effects than any obtainable by the classical method of applying the biuret test, and exceptional economy of all necessary materials was assured by its employment. After ascertaining that the blue color in the coppered caustic alkali solution was not due to a finely divided suspension, we felt that the last objection to the selection of 4 oz. bottles as our reagent containers had disappeared, and to each set of 32 bottles was added one bottle labeled "BIURET REAGENT."

The routine use of the biuret reagent was inaugurated in our laboratory of physiological chemistry at Teachers College in the fall of 1909. Its employment there and in the other laboratories of the department has been proceeding continuously, with perfect satisfaction. Two years ago the senior author published a preliminary statement regarding the utility of his reagent.³ Its continued use in the laboratories of this department has emphasized both the practical and the scientific value of the biuret reagent from every point of view.

During the summer of 1910 the junior author cooperated in a detailed study of the biuret reagent from a number of practical standpoints, especially with regard to the influence of substances and conditions which occur in connection with every day applications of the biuret test. A preliminary report of some of the results has already been published.⁴ Our findings made it evident that the biuret reagent affords, in general, a more effective means of applying the biuret test than any other hitherto described. The employment of the biuret reagent saves time, economizes material, prevents the introduction of an excess of copper, affords a satisfactory color

³Gies: Proceedings of the American Society of Biological Chemists, 1910, i, p. 273; Journal of Biological Chemistry, 1910, vii, p. lx.

⁴Kantor and Gies: Proceedings of the American Society of Biological Chemists, 1911, ii, p. 11; Journal of Biological Chemistry, 1911, ix, p. xvii.

control in doubtful cases, and supplies a stock solution for convenient use in comparative tinctorial tests. We know of nothing which interferes with successful application of the biuret reagent that does not have the same or even more deleterious effect on the biuret test as applied in the classical manner.

In our second preliminary report on this subject we made the following remark: "*The reagent is useful for the detection of reducing substances.*"⁵ In this respect the biuret reagent provides the conditions that prevail in the Trommer test. The biuret reagent is not as satisfactory for this purpose as the Fehling-Benedict reagent, evidently because the concentration of the alkali is too high and the concentration of the copper is too low. (See the second footnote, page 265.)

Among our findings was the observation that although ammonium salts interfere with protein responses to the biuret reagent, ammonium hydroxid fails to do so to any appreciable degree. Ammonium salts react with the alkali of the reagent. By reducing the alkalinity of the liquid not by introducing ammonia into it, ammonium salts require the addition of a moderate excess of the reagent for an elicitation of the test⁶ in any protein mixture containing any of them in a fairly large proportion.

During the past summer, in reflecting on the results with ammonium salts and ammonium hydroxid to which we have just referred, the senior author recalled his first quantitative experiences with the Pavy reagent,⁷ and thought of the possibility of using decolorized Pavy reagent as a *colorless* biuret reagent. As is well known, the Pavy reagent is, in effect, Fehling reagent rendered strongly ammoniacal. By careful reduction, with dilute glucose solution for example, the resultant cuprous oxid may be kept in solution, the copper combination with ammonium hydroxid being colorless although speedy oxidation to a blue compound is difficult to prevent. Experiments in this connection were performed immediately after the idea came to mind. The very first test revealed the

⁵ Kantor and Gies: loc. cit.

⁶ The details of our work in all these connections will soon be published elsewhere.

⁷ Asher and Gies: *Zeitschrift für Biologie*, 1900, xl, p. 180.

fact that colorless (reduced) Pavy reagent is a very effective substitute for the biuret reagent in the biuret test. Each of many tests confirmed this finding. The tendency to prompt restoration of the blue color, unless we devise means of preventing it, makes it necessary to prepare fresh portions of this colorless reagent frequently for its satisfactory general employment.

The biuret reagent itself is easily reduced to a practically colorless condition by heating it with a proper amount of very dilute glucose solution, but cuprous oxid may then be seen in it. If, however, the biuret reagent is rendered ammoniacal and then cautiously reduced to the colorless condition with dilute glucose solution, the resultant colorless liquid is free from cuprous oxid and with it protein gives the biuret test beautifully. The keeping qualities of the decolorized biuret reagent are better than those of the reduced Pavy reagent, evidently because of the lower concentration of copper in the former.

These observations naturally led to the trial of an "*alkaline reducing agent*." Zinc dust, the only reducing agent of this kind that it has been convenient to try thus far, promptly reduces the biuret reagent to the colorless condition. In this state the reagent produces the biuret coloration in a protein solution but the reagent is less effective than before its decolorization. Many more experiments than the few we have been able to find time to conduct will be required, in connection with methods of reduction, before we shall be satisfied that a permanently colorless, perfectly satisfactory, biuret reagent cannot be prepared by such means.

Thus far the most satisfactory attempts to prepare a colorless biuret reagent have depended upon (1) the solution of cuprous oxid in ammonium hydroxid solution under conditions that prevent oxidation, and (2) the addition of caustic alkali to this colorless ammoniacal solution in sufficient proportion to accomplish effective disorganization of protein for the maximum production of typical biuret coloration. We hope to extend these particular experiments, and others collateral to them, at an early opportunity.

The chief difficulties in the way of satisfactory preparation of a colorless biuret reagent have been (1) the persistent tendency of the colorless ammoniacal reagents to acquire color spontaneously or

(2) the removal of much of the copper from solution in the only metallic reduction process thus far attempted.

While the preparation of a colorless biuret reagent is theoretically desirable, it is quite probable that a colorless reagent would fail to *exceed* the blue biuret reagent in *effectiveness*. The color contrast between the blue reagent and the red product with protein appears to be helpful in forming a conclusion in difficult cases. Thus far the original biuret reagent has met every expectation in tests for the smallest proportions of protein that can be detected with the aid of the biuret test as applied in the classical way and, pending the successful preparation of a satisfactory colorless reagent, if we ever succeed in devising it, we strongly recommend the blue reagent.

The biuret reagent may be prepared in the following proportions: Into 1000 c.c. of 10 per cent. sodium hydroxid solution pour 25 c.c. of 3 per cent. cupric sulfate solution, a few cubic centimeters at a time, with vigorous shaking after each addition. The beautiful blue solution is the reagent. Weaker or stronger copper solutions may be made, by changing the volume of added copper sulfate solution, but our experience indicates that, for routine work, the proportions indicated above are as satisfactory as any. These solutions keep indefinitely, but silicate, ferric hydroxid and other materials may form sediments, if the chemicals are impure or the walls of the container are attacked.

If filtration of the biuret reagent is desirable at any time, glass wool may be used to accomplish it. *Filter paper and cotton wool cannot be employed for this purpose* because of the attraction between cellulose and the copper of the reagent, a fact to which we have already made public allusion and to which we intend to return with details at an early date.⁸

⁸Kantor and Gies: loc. cit. We have already published our observation of the fact that when filter-paper strips are suspended in the biuret reagent, the paper "absorbs" the copper, is colored blue as a consequence and the reagent itself is decolorized. We have lately observed the same blue coloration of filter paper strips suspended in *colorless* ammoniacal biuret reagent.

EDEMA

Abstracts of the communications which comprised the symposium on edema at the second scientific meeting of the Columbia University Biochemical Association, held at the College of Physicians and Surgeons, on June 5, 1911

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I. INTRODUCTION, WITH REMARKS ON LYMPH AND ITS RELATION TO EDEMA

William J. Gies

Introduction. Edema is a pathological condition. It is characterized by an abnormal accumulation of water in a part, or in the whole, of an organism. Great distention may result. Edema may occur in any organism, living or dead. Local edemas are clinically designated by special terms, such as ascites, hydrocele, hydrocephalus, glaucoma. In most cases these terms indicate specifically the location of a particular edema.

The water of edema in a given tissue is both intracellular and extracellular in occurrence. The water in edematous tissue appears to be combined, or intimately associated, in large part, with both intracellular and interstitial *colloids*. The accumulated excess of water in an edematous tissue naturally carries dissolved substances with it, but water accumulates disproportionately, in some respects,

in edema. The pathological conditions attending the development of edema effect the transference of abnormally large and unwieldy volumes of intravascular water to extravascular positions, or are responsible for special interferences with the normal movement of extravascular water into the circulation, or induce both of these general results.

The mechanical and chemical factors involved in the localizations of water which are characteristic of edema, have long been subjects of experimental inquiry and earnest discussion. It has generally been assumed that the excess of water in an edematous tissue represents, in effect, an abnormal relation between lymph production and lymph removal (absorption), *i. e.*, that edema is *pathological* lymph formation, collection, and retention. For this reason practical study of the presumed causes of edema has been repeatedly directed to an extension of our knowledge of, and to reexaminations of the factors and influences involved in, *physiological* lymph formation. In recent years, however, stress has been laid upon local tissue disturbances, such as deficient intracellular oxidations, as the causal influences in the development of edema. (See page 279.) Such intracellular conditions would exert hydrophilic influences directive of and coordinate with, *but not independent of*, the primary phenomena in physiological lymph production.

Lymph and its relation to edema. The foregoing statements indicate that edema, in the *prevalent* view, is a pathological accumulation of lymph. Let us consider for a few minutes our general knowledge of the mechanical factors in the physiological formation of lymph.

Blood circulates, under pressure, to all living parts of the body. Plasma (water with substances dissolved in it) passes through the thin walls of the blood capillaries into the tissue spaces and becomes (mingles with) extravascular (interstitial) lymph. Under normal conditions all the cells—all the “tissue elements”—are continuously bathed with flowing lymph. Interstitial lymph carries oxygen, water, nutrients and stimulants to the cells and, in exchange, it receives the expelled products of intracellular metabolism. Interstitial lymph (water with substances dissolved in it) passes through

the thin walls of blood capillaries, and also into lymph channels, and through the thin walls of lymph capillaries, thus becoming directly, and by way of the lymphatic trunks, a part of the circulating blood. In a normal tissue there is practical equality between the total income of plasma and the total outgo of lymph. In an edematous tissue this equilibrium is modified—a balance accumulates in favor of income (and retention) of lymph (water).

If these statements are correct, and if edema is simply pathological lymph accumulation, we should expect to find that edema in any tissue results from inadequate removal of lymph from the tissue spaces to the circulation, or from inordinate transudation of plasma from the blood to the tissue spaces, or from both conditions.

Physiologists are not in agreement in their views on the exact nature, and the relative importance, of all the influences and factors which induce lymph formation. The following summary indicates the powers, processes, and properties which have long been regarded as the dominant influences in the passage of blood plasma to the tissue spaces and in its compensatory return as lymph to the circulation:

I. FACTORS WHICH DIRECTLY OR INDIRECTLY FAVOR THE CONTINUOUS PASSAGE OF BLOOD PLASMA (WATER AND DISSOLVED SUBSTANCES) TO THE TISSUE SPACES.

A. *Conditions which directly or indirectly facilitate filtration, diffusion, or osmosis, or all of these processes.*

- (a) Permeability of the walls of the blood capillaries.
- (b) High pressures in the arterial blood capillaries (as compared with those in the tissue spaces).
- (c) Low pressures in the tissue spaces (as compared with those in the blood capillaries).
- (d) Renewal (from supplies in the alimentary tract) of water and plasma constituents in the blood.
- (e) Outward movement of lymph from the tissue spaces into lymph channels and blood capillaries.

B. *Special cellular influences in lymph production.*

- (a) Secretory activity of the endothelium of the blood capillaries.
- (b) Active intracellular utilization of, and demand for, plasma constituents (water and nutrients).

II. FACTORS WHICH DIRECTLY OR INDIRECTLY FACILITATE THE CONTINUOUS PASSAGE OF LYMPH (WATER AND DISSOLVED SUBSTANCES) FROM THE TISSUE SPACES TO THE BLOOD.

A. *Conditions which directly or indirectly facilitate filtration, diffusion, or osmosis, or all of these processes.*

- (a) Permeability of the walls of the capillaries.
- (b) Low pressures in the lymph capillaries and venous circulation (as compared with those in the tissue spaces).
- (c) High pressures in the tissue spaces (as compared with those in the lymph capillaries and venous circulation).
- (d) Excretion from the blood of diffusible substances brought from the cells.
- (e) Inward movement of plasma from the blood capillaries into the tissue spaces.

B. *Special cellular influences in lymph removal.*

- (a) Secretory activity of the endothelium of the lymph capillaries.
- (b) Active intracellular production and ejection of products arising in tissue metabolism.

The above-named factors, or some of them, exert influences which, under ordinary conditions, maintain, in any tissue, the normal relations of lymph production and removal. Recent observations suggest that other factors, among them colloidal hydrophilia, cooperate in effecting the normal relationships (p. 279). It is obvious that disequilibration of the normal coordination of forces would tend to yield an abnormal result in any tissue, either in the direction of excessive lymph production or of deficiency in its removal, or of both.

In a recent discussion of "the distribution of solutions in cardiectomized frogs," Meltzer emphasizes his views on the relation of the flow of liquids in the "tissue spaces" in the following terms:¹ "Finally, the service of the peripheral mechanism may be called into play also when the general circulation, for one reason or another, becomes inefficient; the excess of lymph which is not carried off by the blood capillaries and the lymphatics is then carried further

¹ Meltzer: *Journal of Experimental Medicine*, 1911, xiii, p. 556.

through the system of tissue spaces. When the impediment of the circulation is too great and the system of tissue spaces becomes overloaded with lymph, we have then the picture of general edema and anasarca."

The appended summaries suggest the causes of edema as Cohnstein and Starling estimate them. These two summaries emphasize the possibilities in disturbed equilibrium of the normal forces in lymph production and removal, but they also suggest the current views on the intimate relationship between the normal formation of lymph and its pathological modifications in quality and quantity.²

COHNSTEIN'S SUMMARY OF THE CAUSES OF EDEMA

- I. Increase of intracapillary pressure.
- II. Reduction of pressure within the tissues.
- III. Increase of permeability of the capillary wall.
- IV. Chemical changes in the blood plasma.
- V. Chemical changes in the tissue fluids.
- VI. Disorders in the removal of the tissue fluids by the lymphatics.
- VII. Disorders in the removal of the tissue fluids by the blood vessels.

STARLING'S SUMMARY OF THE CAUSES OF EDEMA

I. Factors causing increased transudation.

A. Increased intracapillary pressure.

- (a) Venous obstruction.
- (b) Vasodilation.
- (c) Plethora.

B. Increased permeability of vessel-walls.

- (a) Local injury by mechanical irritants.
Local injury by thermal irritants.
Local injury by chemical irritants.
- (b) Malnutrition.
- (c) General injury by circulating poisons (?).

²The reader is referred to Meltzer's very complete critical review of the "physiologic and pathologic factors concerned in the formation of edema." Meltzer: Harrington Lectures on Edema (University of Buffalo, N. Y.). American Medicine, 1904, viii, pp. 19-23, 59-63, 151-155, and 191-199. The summaries credited above to Cohnstein and Starling have been taken from Meltzer's splendid review.

C. Watery condition of the blood (hydremia).

II. *Factors causing diminished absorption.*

A. By lymphatics.

(a) Paralysis of limbs.

(b) Obstruction of lymphatic trunks.

B. By veins.

(a) Venous obstruction.

(b) Watery condition of the blood.

(c) Concentrated transudations.

Fischer's collochemical theory of edema is discussed in section III of this symposium (p. 279).

II. THE EARLIER THEORIES OF EDEMA

Jacob Rosenbloom

In a brief discussion of this subject, which is very comprehensive, it is desirable to commence with the theory of Richard Bright who, in 1827, published his views on edema. He believed that edema was due to blood poor in albumin. The "thinned" blood in his view reached the tissues by filtration while the normal blood was too thick to pass through the vessel walls. It is interesting to note that this theory held sway for about 50 years. Bartels proposed a modification of Bright's theory by suggesting that the loss of albumin was not the cause of edema but only a favorable factor for an easy transudation of fluid constituents of the blood. In Bartel's view edema was due to a retention of water in the blood leading to a thinned condition of the serum and a correspondingly increased amount of water in other fluids of the body, filtration playing the principal part in the transudation process. Ludwig's work (1850) on the secretion of saliva showed that the driving force of secretion was inside the cell and not outside. Heidenhain's work (1891) on absorption and on flow of lymph showed that neither could be explained upon the basis of filtration and diffusion alone.

In order to test the theory of Bright and Bartels, Cohnheim and Lichtheim (1877) injected intravenously 6 per cent. solutions of sodium chlorid into dogs and found that although an amount of salt equivalent to over 60 per cent. of the weight of the dog

could be thus administered without a fatal result, anasarca never ensued. However, some of the organs were edematous and in a few dogs ascites was present. The organs showing edema in nephritic conditions were never so affected by injected salt. Because they were able to produce anasarca in dogs by subjecting the part to irritants before the salt solutions were injected, Cohnheim and Lichtheim concluded that an alteration of the vessel walls was necessary for the passage of fluid. These investigations disproved Bright's theory.

Gaertner (1891) repeated the experiments of Cohnheim and Lichtheim but frequently obtained different results. He observed extensive edema of the skin and underlying tissue, and claimed that the results published by Cohnheim and Lichtheim were due to a too rapid injection of the salt solution. Gaertner, with Bartels, Stewart, and Garinger, maintained that the principal cause of edema lay in an excessive amount of water in the system.

Fleischer, repeating the experiments of Cohnheim and Lichtheim, injected salt solution after ligating the ureters and found neither increase in the blood pressure nor production of edema of the skin. Francotte (1888) in similar experiments, found that edema of the skin followed such injections.

Lassar (1877) observed that the amount of lymph coming from a lymph vessel of an *inflamed* extremity was greater than normal. Emminghaus (1874) found this to be the case, also, in obstruction edema, but there was a great deal of difference between the physical and chemical properties of fluids drawn from a leg which was inflamed and from one in which stasis existed. These facts indicate that conditions which change the permeability of the vessel walls have much to do with the causation of edema.

Welch (1878) made an extended study of the production of edema of the lungs by ligating the arch of the aorta and the left subclavian artery. When he tried to produce edema of the lung by venous obstruction it was necessary to ligate all the veins of the left lung and those of the upper lobe of the right lung. He concluded that an explanation of edema of the lungs upon the basis of active and passive hyperemia was insufficient. In other experiments he produced edema of the lungs by digital compression of the left

ventricle, thus hindering its action and producing a disproportion between the action of the right and left ventricles.

Mayer soon made similar experiments with the same results. He thought that this type of edema is due to an obstruction to the return of blood from the lungs and by the driving of blood into the organ by accessory forces such as (1) increased tone of the vessel wall, (2) increased pumping force of the thorax, and (3) spasm of muscle, especially the diaphragm and abdominal muscles. Colin (1883) repeated the work with the same results and found that the vasomotor nerves played an unimportant part, while Ranvier (1869) held that vasomotor paralysis was a necessary factor.

Landerer (1884) claimed that alterations in the elasticity of the tissues is the primary factor in the change of the circulation of the blood and lymph, and that edema is the result of an impairment of the elastic equilibrium of the circulating fluids of the body.

Salvioli (1885) confirmed all of Cohnheim's results but suggested no other influence than filtration in the production of edema. He thought that transudation was dependent upon the nutrition of the animal, the concentration of the injected fluid, and the pressure. The principal cause of edema in Salvioli's view is the permeability of the vessel walls, which he tried to explain upon a purely mechanical basis.

Von Basch considered that edema of the lung was caused by the rigidity of the walls of the alveoli as induced by pulmonary hyperemia, the diminished elasticity of the tissue giving rise to edema.

Sahli (1884) denied that edema of the lungs is due to an obstruction. He believed that impaired action of the left auricle or spasm of heart muscle can never cause the increase of blood pressure in the circulation of the lungs which is necessary for the production of edema. He considered that edema was due to local alteration of the capillary walls.

Dickenson (1893) investigated the question whether filtration or diffusion played the principal rôle in edema. He concluded that diffusion is the principal factor in the interchange of salts, while blood pressure determines the escape of albuminous con-

stituents of the blood. Senator's (1868) experimental results led him to advocate the theory of Cohnheim. Hamburger (1895) also supports Cohnheim's theory.

It is obvious from this brief review, that for about 100 years there have been two main ideas on the cause of edema: First, changes in the character of the blood; second, changes in the permeability of the vessel walls. While no doubt both of these influences are important factors in edema, the parts played by the tissues themselves must not be overlooked.

Loeb (1898) claimed that the tendency to transudation of fluid into the tissues in edema is greater than that into the blood and lymph, and that therefore the cause of edema must be either an increase in the osmotic pressure of the affected tissue or a decrease of the osmotic pressure in the circulatory system. Loeb demonstrated the possibility of a rapid change in the osmotic pressure of tissues, thus showing that the forces which (according to the filtration theory) are able to produce edema are wholly inadequate. The osmotic pressure of 0.7 per cent. sodium chlorid solution amounts to 4.9 atmospheres, which is twenty times more than the blood pressure. Only a trace of acid or alkali in muscle increases the osmotic pressure to such an extent that the muscle becomes edematous in 0.7 per cent. salt solution. By tetanizing muscle, its osmotic pressure increases one atmosphere and through the original loss of water, chemical changes take place in the muscle which raise its osmotic pressure twenty-five atmospheres. Welch never obtained a rise of more than $1/10$ of an atmosphere, so it is easily seen how much more powerful the change in the osmotic pressure is, as compared with circulatory changes. Loeb thinks that blood and epithelial cells are present in edematous fluids as a result of morphologic changes in the blood vessels. He believes these changes are brought about by the same poisons which produce the increase in osmotic pressure and has shown that these substances may be produced by a lack of oxygen.

In order to test the filtration theory Loeb made the following experiment: The circulation in a hind leg of a frog was stopped by means of a ligature. The ligatured extremity, when immersed in water, increased in weight: after 16 hours, 2-3 per cent.; after

24 hours, 15 per cent.; after 7 days, 25-40 per cent. Similar results were obtained in many experiments. In order that there might be no possibility of an increase in blood pressure in spite of the ligation, the muscles were cut from the frog and placed in salt solution. By this means there was no question of blood pressure and a lack of oxygen was also produced. These muscles behaved like the ligatured muscles, showing conclusively that blood pressure had nothing to do with the edema produced. Loeb made many other experiments and *came to the conclusion that edema is caused by chemical changes which occur in the tissues and which are mostly due to a lack of oxygen.* These changes lead to an increase in osmotic pressure, which is greater in the tissues than in the blood and lymph, and therefore the tissues take up fluid and become edematous. This explanation seems very plausible and also accounts for the sodium chloride retention of the French school and the various kinds of local edema.

(Fischer's extension of Loeb's theory of edema is the subject of the discussion to follow.)

This brief sketch of the development of our ideas concerning the cause of edema, emphasizes the fact that the farther we progress in our knowledge of physiologic and pathologic phenomena, the more we find that the ultimate causes reside in the tissues themselves. Since tissue morphology and function are nothing but questions of the nature and powers of the cells that compose a tissue, it is obvious that there is great need for a thorough knowledge of the chemical statics and dynamics of individual cells. It is in this field that great advances must be made for the elucidation of many physiological and pathological problems.

III. FISCHER'S THEORY OF EDEMA

William J. Gies

The most important recent contribution to our knowledge of edema has been made by Fischer in an elaborate extension of the work and views that were published by Loeb several years ago (p. 278).

General statement. Fischer's theory of edema is summed up in his recent book on the subject as follows:¹

A state of edema is induced whenever, in the presence of an adequate supply of water, the affinity of the colloids of the tissues for water is increased above that which we are pleased to call normal. The accumulation of acids within the tissues brought about either through their abnormal production, or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible for this increase in the affinity of the colloids for water, though the possibility of explaining at least some of the increased affinity for water through the production or accumulation of substances which affect the colloids in a way similar to acids or through the conversion of colloids having but little affinity for water into such as have a greater affinity, must also be borne in mind. (Page 99.)

Preliminary observations. The first of his series of experiments, and one of his fundamental observations, is thus described by Fischer:

The cause of edema resides in the tissues. A very simple experiment proves this fact. If one leg of an ordinary frog (*Rana*), a tree frog (*Hyla*), or a toad (*Bufo*), is ligated just above the knee as tightly as possible, so that the ligature shuts off not only the venous flow, but also the arterial, and the animal is then placed in sufficient distilled water to cover the legs, the ligated leg develops an intense edema, while the unligated one remains normal. To explain this result recourse cannot be had in this experiment to the pressure of any circulating liquids, for none such exists, and so all the conceptions of edema which regard the pressure, per se, of circulating liquids, as one of the causes, or the chief cause, in the development of this condition, are robbed of their most fundamental support. (Page 11.)

At the conclusion of his description of several such experiments Fischer adds:

It is clear that the cause of edema resides in the tissues themselves, and that these become edematous not because water is forced into them,

¹Fischer: Edema—a study of the physiology and the pathology of water absorption by the living organism, being the 1909 Nathan Lewis Hatfield Prize Essay of the College of Physicians of Philadelphia. 1910. Pp. 209. Wiley & Sons, New York. A preliminary statement of his theory, and the experimental findings on which he bases it, were published by Fischer in *Kolloidchemische Beihefte (Ergänzungshefte zur Kolloid-Zeitschrift)*, 1909-1910, i, p. 93.

but because changes take place in them whereby they are enabled to absorb water from any available source. In the case of the experiments on toads and frogs this available source of water is the water contained in the dishes in which the animals are kept. In clinical cases of edema, this is found in the fluids which pass through or about a tissue. (Page 18.)

As he proceeds in his book with the fortification of his theory of edema, Fischer describes experiments on the swelling of fibrin and gelatin in solutions of acids and alkalies, and also in such solutions that contained or which were free from electrolytes or non-electrolytes. The bloating effects of acid solutions are especially emphasized and the counteracting influences of electrolytes are noted. Thus, in discussing effects on fibrin he says (p. 24): "The addition of any salt to the solution of an acid or an alkali decreases the amount that fibrin will swell in that solution. . . . The higher the concentration of the added salt, the less does the fibrin swell, and if enough is added, the effect of the acid or alkali may be suppressed entirely." Fischer alludes as follows to the significance of these particular experimental findings:

These differences and similarities in the behavior of different colloids toward the same external conditions demand detailed study, for they are of the utmost biological importance. Protoplasm consists, as is well known, of a mixture of many different colloids. Not only are different colloids found in the same cell, but essentially different colloids form the basis of different tissues (bone, cartilage, muscle, connective tissue, parenchymatous organs, central nervous system). It is at once apparent therefore, that not only so far as water absorption and secretion is concerned, but so far as any physiological reaction dependent upon the colloidal constitution of living matter is concerned, a single variation in internal or external conditions may be followed by quite a different response either qualitatively or quantitatively, not only by different tissues but by different parts of the same tissue or even the same cell. In a study of the behavior of different colloids toward the same group of external conditions we may therefore hope to discover much to aid us in our attempt to analyze the apparently limitless variations in the reactions of protoplasm to various external "stimuli." (Page 55.)

The foregoing paragraph is followed by this general statement, under the heading "The analogy between the swelling of certain colloids and the swelling of protoplasm":

Having become familiar with the effect of various external conditions on the swelling of two simple so-called hydrophilic or emulsion colloids (fibrin and gelatin), we have now at our disposal some facts which we may utilize in an attempt to analyze the ways and means by which tissues hold their normal amount of water, and to discover how under altered external conditions they may come to hold more or less than is considered normal. It is clearly evident that could we show that the same conditions which make fibrin or gelatin take up and give off water affect protoplasm similarly, a real step forward in the solution of this problem of the absorption and secretion of water by the tissues would be made. This can be done and with great simplicity. As the following paragraphs show, the absorption of water by muscle or the absorption of water by the eyeball is entirely analogous to the absorption of water by fibrin or by gelatin. (Page 56.)

From the results of his experiments in the latter connections Fischer draws the following conclusions:

The absorption of water by muscle is determined, in the main, by the state of the colloids contained in the muscle. . . . The absorption of water by the eye is governed by the same laws as the absorption of water by fibrin or gelatin. (Page 74.)

Discussing "the biological significance of the analogy between the absorption of water by certain colloids and the absorption of water by muscle and the eye," Fischer amplifies his views by alluding, in the following terms, to the "pressure theory," the "osmotic theory" and to cell "membranes":

We need not here again point out the fatal argument against the pressure theory contained in the observations which show that enormous amounts of water may be absorbed in the entire absence of a circulation—a muscle absorbing more than twice its weight of water in a dilute acid; and a beef eye, enough to rupture the enormously thick and tough sclera. The forces displayed here are so great that they cannot even be approximated by simple blood pressure. (Page 87.)

If the cells obey the laws of osmotic pressure, then it is demanded that in solutions of different substances having the same osmotic pressure the volume of the cells shall be the same. Exceptions to this conclusion are the *rule* with cells. . . . There is little reason for accepting the osmotic theory as of paramount or even great importance in the explanation of the ways and means by which tissues absorb or secrete water. (Pages 90 and 91.)

We will encounter no difficulty in explaining the various experimental facts at our disposal by ignoring altogether the existence of impermeable or partially permeable cell membranes and simply remembering that the substance of a cell consists of a mixture of different colloids. A part of these are colloidal solutions of the proteins with physical and chemical properties analogous to the physical and chemical properties of fibrin, gelatin, etc.; a part, colloidal solutions of the lipoids, which while sharing some of the properties possessed by the protein colloidal solutions, such as their power of swelling in water, have specific properties of their own, such as their power of taking up substances soluble only in the fat-like bodies.² (Page 96.)

In order to complete my argument let me add at once that we cannot and must not consider the absorption, or secretion, of water and the absorption, or secretion, of a substance dissolved in the water as identical processes. Workers in biology make this mistake constantly. The processes of the absorption of water and the absorption of dissolved substances do not parallel each other in simple physico-chemical experiments, and so need not, and do not, in living cells. The two are frequently associated, and may at times lie so closely together that they give the impression of running parallel with each other. (Page 96.)

When we consider protoplasm simply as a mixture of different colloids (proteins, fats, carbohydrates), and consider the special characteristics of absorption that arise out of such a mixture not only as regards water, but as regards substances dissolved, or pseudo-dissolved (colloids) in it, it seems to me that we can account without difficulty, even without membranes, for all those phenomena which have up to the present been interpreted through the assumption of semi-permeable, partially permeable, and lipoidal membranes about cells. (Page 98.)

The nature of Fischer's evidence. Fischer assembles his "proof for the truth of his contention" (as stated in general terms in the opening paragraph of this review), in direct support of the following three theses (p. 99):

1. An abnormal production or accumulation of acids, or conditions predisposing thereto, exist in all states in which we encounter the development of an edema. (Pages 99-109.)

2. The development of an edema in tissues is antagonized by the same substances which decrease the affinity of the (hydrophilic) emul-

² The role of the colloidal carbohydrates is ignored in this discussion simply because very little of immediate interest to us has yet been done with them.

sion colloids for water (salts) and is unaffected by the presence of substances which do not do this (non-electrolytes). (Pages 110-121.)

3. Any chemical means by which we render possible the abnormal production or accumulation of acids in the tissues is accompanied by an edema. (Pages 121-126.)

Edema results from an abnormal production or accumulation of acids. In his consideration of the first of these three themes (pp. 99-109) Fischer discusses the general edemas which develop in conjunction with circulatory disturbances (p. 99), anemias (p. 102), inanition (p. 103), fever (p. 104), nephritis (p. 105), and post mortem influences (p. 107); also local edemas in infarcted areas (p. 107), in gangrenes (p. 108) and from the bites or stings of insects (p. 108). The main lines of his "proof" for his theory of edema causation are indicated by the following selected quotations:

We will have no difficulty in interpreting the variations in the severity of a local or a general edema due to a circulatory disturbance when we say that every condition that makes for a state of lack of oxygen in the edematous parts, be this through disturbances in the affected parts themselves, or in some distant organ, as the heart, makes for an increase in the severity of the edema. This brings us face to face with the following question: Is every state of lack of oxygen accompanied by an abnormal production or accumulation of acid? for, as already stated, this is what we need and know from our previous experiments to be most potent in increasing the affinity of the tissue colloids for water. (Page 100.)

To answer this question we will introduce the striking experimental findings of Trasaburo Araki.³ This author has shown that in lack of oxygen, no matter how produced, dogs, rabbits, and frogs excrete lactic acid in their urine in addition to various other abnormal substances. Under ordinary circumstances this lactic acid is not to be found in the renal secretions, but let the oxygen supply to these animals be sufficiently interfered with, by any means whatsoever (through confinement in a closed box, through carbon monoxide poisoning, or through the injection of curare, morphine, amyl nitrite, or cocaine), and the acid appears. Lest the objection be raised that these remarks may hold for various animals, but need not necessarily be true for human beings,

³ Araki: *Zeitschrift für physiologische Chemie*, 1891, xv, pp. 335 and 546; *ibid.*, 1894, xix, p. 422; see also Hoppe-Seyler, *ibid.*, 1894, xix, p. 476.

let it be noted that Araki found lactic acid in the urine of epileptics voided shortly after their seizures. As a proof that lactic acid is not necessarily the only acid that may be or is produced in states of lack of oxygen, we can mention E. Mendel's⁴ finding that the phosphoric acid content of the urine is increased immediately after epileptic seizures and in apoplexy. We may also call to mind Hoppe-Seyler's analysis of various edema fluids which he found to contain, besides lactic acid, valerianic, succinic, and butyric acids. (Page 101.)

The lactic acid found in conditions associated with a lack of oxygen is produced in the tissues, enters the blood, and is excreted by the kidneys. This has been proved by Araki's later publications and through Hermann Zillessen's⁵ experiments. Zillessen found that when the oxygen supply to a muscle or the liver is shut off for a variable number of hours through ligature of the arteries supplying these parts, an increased production of lactic acid occurs. If the ligature is loosened and the first blood returning from the oxygen-starved tissues is analyzed, this is found to be particularly rich in lactic acid, and if the blood is titrated, it is found to have a diminished capacity for neutralizing a standard oxalic acid solution.⁶ Zillessen was also able to demonstrate the production of lactic acid in animals poisoned with hydrocyanic acid, which we know from Geppert's studies to owe its action to its power of inducing a state of lack of oxygen in the tissues. (Page 101.)

The accumulation of carbon dioxide in the tissues is also of importance. The ordinary circulatory disturbances while making for a decreased supply of oxygen to the tissues also make for an accumulation of carbon dioxide. In experiments with fibrin which I placed in distilled water in ordinary "soda" bottles, and then had charged with carbon dioxide at various pressures in a local mineral water establishment, I found a marked increase in the amount of the swelling with every increase in the concentration of the carbon dioxide. The observation of Strassburg and Ewald, that the carbon dioxide content of edema fluids and of tissues deprived of a circulation runs very high, is therefore not to be disregarded in trying to find a cause for the increased affinity of the tissues for water in a state of disturbed circulation. (Page 102.)

⁴ Mendel: *Archiv für Psychiatrie und Nervenkrankheiten*, iii, p. 636.

⁵ Zillessen: *Zeitschrift für physiologische Chemie*, 1891, xv, p. 387.

⁶ Araki, Zillessen, and most of the older observers speak of a "decreased alkalinity" of the blood. Since modern physico-chemical measurements have shown the blood to be neutral in reaction, it is best to state the experimental findings of these authors as above.

We learn from Araki and Zillessen's observations that the production of lactic acid occurs in animals no matter how the condition of lack of oxygen is induced. In their experiments they used all kinds of methods to induce a lack of oxygen, varying from those which act through direct interference with the oxygen supply to the animal (compression of trachea) to those which we know owe their effect to an action upon the oxidizing ferments of the tissues themselves (hydrocyanic acid). It is of much interest, therefore, that A. Jolles and Oppenheim,⁷ and in the United States, M. C. Winternitz and J. C. Meloy,⁸ have found substances present in the blood of nephritics which interfere with at least some of the oxidation phenomena which we know are necessary for the proper continuance of life. (Page 106.)

The local edemas following the bites or stings of insects have a special interest. In quite a number of these the sting carries formic or other acids into the tissues. Here we have a direct etiological factor for the production of the local edema. In others, poisons are injected which have a well-marked reducing power. By this means a local group of cells is placed in a state of lack of oxygen through chemical means. It is worthy of note that to start with and during the period of greatest swelling such insect stings are white, and not until later do they become red. The increased blood flow so necessary in most explanations of these local edemas does not occur until the edema has begun to subside. Instead of the blood circulation determining the edema, the edema determines whether the circulation shall continue through the affected part or not. (Page 108.)

Edema is antagonized by electrolytes but is not affected by non-electrolytes. In his treatment of this theme (pp. 110-121), Fischer gives the results of numerous experiments "to show that the same conditions which have been found effective in reducing the amount of swelling of fibrin and gelatine in acid solutions counteract the development of edema." Frog legs which have been ligated, cut from the body, and placed in a little water "develop an edema which mimics in every way the worst types of edema observed clinically. We will use the edemas developed in this way in amputated frog legs as material upon which to analyze the nature of the phenomenon."

⁷ Jolles and Oppenheim: *Münchener medizinische Wochenschrift*, 1904, xlvii, p. 2083.

⁸ Winternitz and Meloy: *Journal of Experimental Medicine*, 1908, x, p. 759; also Winternitz, *ibid.*, 1909, xi, p. 200.

Since the formation of acid in tissues deprived of a circulation is a firmly established fact—a fact which in these amputated frogs' legs can be verified through mere application of an indicator—we have no difficulty in interpreting these experiments by saying that the amputated frogs' legs swell in water because the affinity of their colloids is increased through the production in them of acid. The frogs' legs become edematous for the same reason that fibrin swells more in a dilute acid than in pure water. If instead of being placed in distilled water the amputated frogs' legs are dropped into any salt solution, they swell less than in distilled water. The higher the concentration of the salt the less will the frogs' legs swell. These statements are entirely analogous to those made regarding the swelling of fibrin and gelatin in dilute acid solutions. (Page 112.)

The *anions* which were found to be the most effective in *preventing* the swelling of the muscle were those of tartrate and phosphate, whereas the least effective were the bromid and chlorid anions. These results, like the negative effects of non-electrolytes, were in accord with the similar data pertaining to the influence of anions on the swelling of fibrin and gelatin.

Abnormal production or accumulation of acids in the tissues, by chemical means, is accompanied by edema. In his discussion of his third thesis (pp. 121–126), Fischer gives the results of several experiments intended “to prove that any condition which makes for the production of acid in the tissues leads to the development of an edema if a source of water is available” (p. 121). In this connection he says:

The quickest way to put the tissues of an animal into a condition that permits of the development of acids in them is to kill the animal. The fact does not surprise us, therefore, that an edema develops with greater ease in a dead animal than in a living one. If a living frog is kept up to its neck in distilled water it suffers little variation in weight. A change in weight of 3 per cent. covers the extremes. But let the frog be killed and be kept similarly covered with water and a progressive rise in weight at once sets in. (Page 121.)

Fischer states “that the injection of any of the poisons used by Araki into the dorsal lymph sac of a frog is followed by an edema. Frogs poisoned with morphin, strychnin, cocain, arsenic, or uranyl nitrate all absorb amounts of water which run from 15 to 60 per cent. of the normal weight of the frog.” (Page 123.)

Such *chemical edemas* are of more than academic interest. Inasmuch as we find in the list of poisons enumerated above, heart poisons, kidney poisons, nerve poisons, poisons that increase or decrease blood pressure, that increase or decrease lymph flow, that injure bloodvessel walls, or have not been proved to do so, etc., do we not, first of all, seriously question every theory of edema that would establish any one or all of these conditions as the primary cause of all edemas? Secondly, these chemical edemas have interesting clinical parallels. The edema of arsenic poisoning and of poisoning by certain other metals is a well-known condition. We are also able to understand how, after the administration of morphin, chloroform, ether, and alcohol (save in small amounts), a certain degree of edema may develop, or at least a fall in urinary secretion and an increased thirst be noted. All these substances make for a lack of oxygen and an abnormal production of acids in the tissues. At least part of the effects of these various substances may, therefore, be satisfactorily explained through their effect upon the tissues generally, whereby these become edematous. Associated with this there must be a fall in urinary secretion and thirst. A final question of interest in connection with these chemical edemas (with which we must class the edema of kidney disease) is the distribution of the edema in clinical cases. These chemical edemas are always more general, and, as is well known, are particularly liable to first affect the connective tissues of the face, particularly the eyelids. Other things being equal we would expect in a general intoxication those tissues which are most capable of swelling to be the first to give ocular evidence of the existence of an edema. It is interesting, therefore, that of the various tissues examined in this regard I found the connective tissues of the orbit not only to be possessed of colloids most sensitive to low concentrations of acid, but also to have, weight for weight, the greatest affinity ('specific affinity') for water. (Page 126.)

The edema of special organs. Fischer discusses the edema of special organs (pp. 127-155) and the adequacy of his theory for the explanation of glaucoma and edemas of the kidney, liver, and lung.

Enucleated eyes swell and rupture when immersed for a short time in acid solutions of appropriate character and concentration.

The most intense grades of glaucoma can be induced experimentally in an eye in the entire absence of any circulation (p. 127). This increased absorption of water by the eye is dependent upon the colloids in

the eye, for not only is the eye built up of a series of different colloids (sclera, cornea, lens, vitreous humor), but the same conditions which govern the absorption of water by fibrin also govern the absorption of water by the eye. On the ground of these experiments we can, therefore, no longer insist that an eye becomes glaucomatous because water is forced into it. It does this because chemical changes occur within the eye which increase the affinity of the ocular colloids for water so that these are enabled to absorb water from any available source. In our experiments with enucleated eyes this source is the solution into which the eye has been dropped; in the body it is the liquids flowing about or through the eye. (Page 128.)

In harmony with the observations on fibrin, gelatin, and muscle, electrolytes sharply antagonize the swelling of enucleated eyes in the acid solutions employed by Fischer, whereas non-electrolytes are without special restraining effects.

Discussing the passive congestion edemas of the kidney and liver, pp. 141-9, Fischer says:

In the light of our colloidal conceptions of water absorption, how must we interpret the phenomena that characterize the passive congestion edemas of the kidneys and the liver? The cause of the edema is again to be sought in the tissues. The circulatory disturbances leading to an edema of these organs all have this in common: they lead to a state of lack of oxygen in the tissues, in consequence of which acids are produced in them. These acids increase the affinity of the tissue colloids for water, whereby they are enabled to absorb an increased amount of water from any available source. This idea is supported by the following:

It is a well-known fact that when the efferent (renal) vein of the kidney is tied in animals, the organ becomes filled with blood, and that the kidney tissues proper swell and become progressively firmer in consistence. This is the typical picture of a passive congestion sufficiently severe to permit of the development of an edema in the congested area. We need not repeat that what happens in this experiment is usually interpreted as an edema due to an increased blood pressure, alterations in vascular permeability, etc. All these explanations fall as soon as it is stated that ligation of the renal artery leads to the same series of changes in the kidney as ligation of the renal vein (with the exception of the overfilling of the bloodvessels). (Page 142.)

After our remarks on the essential role played by lack of oxygen,

and not mere blood pressure changes in the edema of the kidney, it does not surprise us to recall the well-known fact that ligation of the portal vein is followed by no grossly apparent morphological changes in the liver—the portal vein carries only venous blood to the liver, and so changes in the parenchyma due to the production of acids and a consequent edema of the hepatic tissues is not to be expected. Quite a different picture is obtained when the hepatic artery is ligated. In spite of the fall in blood pressure brought about by this means the liver rapidly develops an intense edema. This result is quite expected on the basis of our theory, and indicates very clearly that the real reason why a passive congestion leads to an edema of the liver is because it interferes with the necessary flow of arterial blood through the organ via the hepatic artery. (Page 144.)

Fischer's views on the cause of edema of the lungs, are stated in part as follows (pp. 149–156):

The problem of pulmonary edema is identical with the problem of the edema of such an organ as the liver. The reason for this is at once apparent when we call to mind the fact that the vascular arrangement in the lungs is very similar to that which we previously discussed for the liver. Just as the liver, so is the lung supplied with two blood streams—with a venous stream through the pulmonary artery, which only passes through the lung for purposes of oxygenation, and an arterial stream through branches from the thoracic aorta, the bronchial arteries, which supplies the parenchyma of the lung with oxygen. The blood brought through these nutrient arteries leaves the lung in part through the bronchial veins, in part admixed with the blood of the lesser circulation through the pulmonary veins. The various facts at hand on the experimental production of pulmonary edema are all easily interpreted as soon as we say that an edema results whenever the oxygen supply to the parenchyma of the lung is sufficiently interfered with. (Page 150.)

This conception of edema can be tested in yet another way. If the lung becomes edematous through any condition which interferes with a normal oxygen supply to the parenchyma, then it ought to be particularly easy to produce an edema in a lung that has been removed from the body. As a matter of fact, *the most intense edemas of the lung which simulate in every way those observed at the autopsy table* may be produced in lungs removed from the body, and in the entire absence of any such blood pressures as are considered active in the current theories of pulmonary edema. (Page 153.)

Turgor, plasmolysis and plasmoptysis. *Turgor, plasmolysis and plasmoptysis* are discussed by Fischer from the standpoint of his experimental observations on water absorption by colloids (pp. 156-180).

It is impossible to escape the experimentally well grounded conclusion that most, if not all, cells do not follow the laws of osmotic pressure. The attempts that have been made to harmonize the observed behavior of various cells with that demanded on the theory that cells represent osmotic systems are ingenious, but we can scarcely believe sufficiently supported by experiment to be convincing. For the most part the explanations given are complicated, which constitutes in itself a threatening feature when the explanation of any natural phenomenon is hazarded. What strikes one as particularly encouraging about the colloidal idea of water absorption is its simplicity, and the breadth of water absorption phenomena to which it may be applied without apparent experimental or theoretical objection. (Page 158.)

The absorption of water by kidney and liver cells is essentially a function of their colloidal state. What was said regarding these cells is also true regarding the behavior of spermatozoa, of white blood corpuscles and of the epithelial cells of the bronchi, intestine, bladder and esophagus. (Page 158.)

All the cells mentioned in this paragraph swell less in any salt solution than in distilled water. With every increase in the concentration of the salt there comes a progressive decrease in the amount of the swelling. At a certain concentration the cells maintain for a variable length of time what is considered their "normal" volume. If the concentration is increased beyond this they shrink. In this brief description are exemplified all that is contained in the terms plasmoptysis, turgor, and plasmolysis as understood by the plant physiologists. Impossible as it is to understand all these phenomena on the basis of osmotic pressure, equally easy is it to see in them a perfect parallel of an emulsion colloid swelling in a dilute acid in the presence of variable amounts of any salt. (Page 159.)

If a frog's muscle is dropped into distilled water it suffers a progressive increase in weight. This phenomenon is usually interpreted as a response to immersion in a solution of too low an osmotic pressure, so that water is absorbed by the cell contents. I maintain that this is not correct, for were it, all our muscles ought to swell whenever we con-

sume a quantity of fresh or distilled water, and a frog living in a fresh water pond ought to do likewise. But this does not occur. Clearly the muscle swells only because removed from the body. The difference between the muscle inside and outside of the body is this: Outside of the body the muscle develops an acid reaction, and in this and its effects upon the muscle colloids I would find the cause for the increased absorption in distilled water. Added to this is the effect of the diffusion of salts out of the muscle, for the higher the concentration of salts in an (hydrophilic) emulsion colloid the less does that colloid swell in a dilute acid. Quite contrary to the generally accepted belief, a loss of the osmotically active electrolytes of a tissue may, therefore, distinctly favor the absorption of water. We will do well to consider this whenever we try to define wherein lies the "poisonous" effect of distilled water. That the extirpated muscle becomes acid in reaction must be borne in mind when we try to interpret the effects of acids, alkalies, and salts upon it. To put a muscle into a dilute acid instead of into distilled water is simply to add the effects of the external acid to that produced spontaneously by the muscle. (Page 161.)

On the *osmotic* conception of water absorption physically "isotonic" solutions ought to be physiologically "isotonic." Yet experimentally this is not found to be the case. On the *colloidal* basis of water absorption this result, of course, does not surprise us, for physically isotonic solutions of different salts are not equally effective in reducing the swelling of an (hydrophilic) emulsion colloid in a dilute acid. (Page 162.)

It is a simple matter, therefore, to account for all the available experimental facts on the absorption of water by muscle on the colloidal basis. Not only are the facts which it has been difficult to harmonize with the osmotic conception of water absorption explained in this way, but all the phenomena which we have been most willing to accept as osmotic may well represent only a fraction of that greater series of phenomena which we have designated colloidal. The entire question of the validity of the laws of osmotic pressure in the biochemistry of water absorption is therefore raised in the special case of muscle just as we have previously raised it in the case of spermatozoa, isolated epithelial cells, and white blood corpuscles. That the laws of osmotic pressure, even as rendered more generally applicable to biological material through Overton's special assumptions, are incapable

of accounting for all the observed biological phenomena, is admitted by this author himself, and in seeking an explanation of various aberrant phenomena he too considers the role of the tissue colloids. He refers, as Pfeffer before him, to the part played by the imbibition water of the cells (*Quellungswasser*), and at one point, correctly to my mind, declares the swelling of muscle in dilute acids to be identical with the swelling of fibrin in dilute acids. But upon this colloidal absorption he does not lay much weight, as is very evident in even his latest writings.⁹ (Page 164.)

It must be clearly understood that this questioning of the rôle of osmotic pressure in biological material so far as water absorption is concerned does not question its importance in the general problem of the diffusion of dissolved substances. This is an entirely separate problem. Only our colloidal conception of water absorption renders possible the diffusion of dissolved substances into regions where on the osmotic conceptions we know they could not get. As already pointed out, neither do our considerations affect the general biological significance of the law of partition as worked out by Hans Meyer and E. Overton in their experimental studies on the cell lipoids. (Page 165.)

When a specific hemolysin or a poison capable of acting at a very low concentration, is added to the blood, the hemoglobin escapes from the corpuscle, but the corpuscle undergoes no change in size. With few exceptions this is not the case in any of the other (hemolytic) solutions—in all of them the red blood corpuscles increase in size when the proper concentration at which hemolysis occurs is reached. Especially marked is this in the solutions of acids and alkalis in which hemolysis occurs very rapidly, and in which swelling is most pronounced (p. 166). I consider changes in the volume of the red blood corpuscles and the loss of hemoglobin by the stroma separate processes, which while they may often be associated, have really nothing to do with each other (p. 168). The red blood corpuscle is essentially a mixture of several colloids—protein (stroma), lecithin, cholesterol and hemoglobin. . . . A class difference, however, exists between hemoglobin and the other colloids that have been enumerated as contained in the red blood corpuscle. Hemoglobin is not a hydrophilic but a hydrophobic colloid; it is not an emulsion colloid (emulsoid) as are the protein constituents of the red blood corpuscle, or lecithin, but a suspension colloid (suspensoid) [p. 169]. The hemoglobin must be combined

⁹ See, for example, Overton's article in Nagel's *Handbuch der Physiologie*, 1907, ii, p. 744 to 896.

in some more or less fixed way with the rest of the corpuscle. The lack of evidence to show that this combination between stroma and hemoglobin is a chemical one, and the fact that an enormous amount of hemoglobin is held by a very small amount of stroma leads me to assume that the combination between the hemoglobin and the rest of the corpuscle represents an adsorption phenomenon (p. 170). The retention and loss of color by carmine-stained fibrin is very similar to, and occurs under the same conditions as, the retention and loss of hemoglobin by the red blood corpuscles (p. 171). The relationships between the different colloids in the case of the red blood corpuscles are, of course, much more complicated than in the case of carmine-colored fibrin. In place of only two colloids, we have in the red blood corpuscles at least four to deal with, and this makes for an infinitely more complicated system (p. 173).

The question to which anyone discussing the general problem of growth (increase of volume) is most desirous of getting an answer is this: What is the source of the energy for growth? . . . The pressures exerted by swelling colloids constitute an adequate source. . . . An absolute *sine qua non* for growth is the presence of water. . . . All growth in volume is preceded by the production of various (hydrophilic or emulsion) colloids. But not only are various colloids produced, but conditions which particularly favor the absorption of water by these colloids are also instituted. It is the rule, for example, that the growing tips of plants have an acid reaction; and the rôle of acids in making various emulsion colloids swell is familiar to us from previous considerations (p. 175). The colloidal conception of water absorption also gives us the means of understanding the mechanism of certain growth curvatures, and curvatures due to tropisms of various kinds as manifested in plants and animals (p. 176). The effect of an increased growth, as evidenced particularly through the presence of an increased amount of water in the convex portion of the plant stem or root, or the animal organism, over that of the convex portion, best explains the observed phenomenon (p. 177). In conclusion, let attention be called to the ready explanation which the colloidal conception of water absorption offers of the ways and means by which it has been found that certain plants and animals protect themselves from a loss of water. . . . When water is scarce certain plants convert some of their starch into oxalic acid. Those types of plants which under natural conditions are most liable to suffer from lack of water (the succulents) seem all to possess the interesting property of reducing their output of carbon dioxide, while

producing at the same time various organic acids as soon as subjected to unfavorable conditions for growth. These phenomena of acid production have been generally interpreted as meaning that by such methods the plant increases the number of soluble molecules in its cell contents and so increases its osmotic pressure. A more correct explanation, it seems to me, is this—through the production of these acids the affinity of the plant colloids for water is increased, so that the agencies operating to rob it of this water are counteracted. A question that awaits an answer in the case of animals is whether a like production of acids is responsible here also for the maintenance of a normal water content, as when a fish, for example, born in fresh water, moves out to sea (p. 179).

On the secretion of urine. The secretion of urine is considered from the viewpoint of the facts of colloidal hydrophilia (p. 180–204). Fischer comments in part as follows on the more important phases of this subject:

It follows as a necessary conclusion from the argument which has been the burden of this paper, that in the resting state the living organism represents a series of different (hydrophilic) emulsion colloids saturated with water. That they are saturated is evidenced by the fact that we cannot make the organism as a whole absorb any more water or give up any without antecedent chemical changes. In consequence, an organism not subject to any marked changes from without or within maintains a constant weight over long periods of time. We need but recall how all the secretions of a man undergoing absolute starvation drop to practically nothing, and how, on the other hand, the consumption of even enormous amounts of water by the normal individual does not lead to the development of the slightest edema. We are accustomed to say that the kidneys quickly rid the body of any excess of water. Just why this is done is not so apparent, though we will suggest an explanation right away. Let it first be pointed out that the blood and lymph constitute an integral part of this water-saturated colloidal system which makes up the body. It may at first sight seem somewhat surprising that the relation between the colloids and the water of liquid (hydrophilic) colloidal solutions (sols) is identical with that of the relation existing between water and solid colloids (gels) such as fibrin. But such an identity is not only demanded by theory but has been proved experimentally by the recent work of Wolfgang Pauli and Hans Handovsky¹⁰ on the blood serum. (Page 184.)

¹⁰ Pauli and Handovsky: *Biochemische Zeitschrift*, 1909, xviii, p. 340.

We are not surprised to find that the secretion of urine ceases (practically) during absolute starvation. If the colloids of the body as a whole are saturated with water, none is left over to be secreted. Only in so far as the tissues undergo gradual consumption during the process of starvation or their colloids suffer changes which decrease their affinity for water is any liberated to become available for secretion (Page 185.)

According to our theoretical considerations on the colloids it would be expected that were a (with-water-saturated hydrophilic) colloidal solution introduced into the vascular system, no increased urinary secretion ought to result. As a matter of fact, it does not, as is proved by the old experiments of Ponfick¹¹ and the more recent ones of Magnus,¹² both of whom found that the injection of blood serum or blood into dogs and rabbits is not followed by a diuresis. (Page 186.)

The simple fact that an amount of urine is always excreted under physiological conditions equivalent to the amount of water consumed tells us nothing, however, of the mechanism by which this is accomplished. To limit ourselves again to the most physiological of the above conditions, let us try to discover how on the colloidal basis of water absorption, water is always absorbed from the gastro-intestinal tract, and always excreted by the kidneys. We can take a step toward the answer to this question by pointing out that the anatomical and physiological conditions existing normally in the body tend to keep the colloids of the gastro-intestinal tract and the blood and lymph streams passing through it, in an unsaturated condition so far as water is concerned, while the reverse conditions hold for the kidney. The mouth and esophagus play practically no role in the absorption of water. The stomach, according to von Mering's experiments, also takes but little if any part in the absorption of water. The small and large intestine are the absorptive organs for this substance par excellence. The stomach is richly supplied with arterial blood. The small and large intestine are also generously supplied, but not as generously as the stomach. The separate branches of the mesenteric arteries which go to supply the villi occupy a fairly central position in this structure and break up into a capillary network which lies close under the intestinal epithelium. As clearly evidenced by the dark color of the portal blood, and direct gas analysis, the blood returning from the intestine is intensely venous

¹¹ Ponfick: *Virchow's Archiv*, 1875, lxii, p. 277.

¹² Magnus: *Archiv für experimentelle Pathologie und Pharmacologie*, 1901, xlv, p. 210.

(poor in oxygen and rich in CO_2). This high carbon dioxide content of the blood returning from the intestinal tract increases the affinity of the colloids of the blood for water in consequence of which they absorb it through the intestinal mucosa from the lumen of the intestine as long as it is present there. The experiments of von Limbeck, Gürber, and Hamburger¹³ show that under the influence of such an increase in CO_2 concentration as exists normally in venous blood over arterial blood the red and white corpuscles absorb an amount of water which easily amounts to from 5 to over 15 per cent.¹⁴ of their volume in arterial blood. If we use only the lower of these values and ignore entirely the water carrying power of the colloids contained in the plasma, a little calculation shows that a liter of blood passing through the intestinal tract is capable of absorbing 17.5 c.c. of water, for the corpuscles when moist make up in round numbers about 35 per cent. of the blood. Even these values, which have been chosen as low as possible, easily suffice to account for the absorption of great amounts of water from the gastro-intestinal tract. (Page 186.)

It requires no special explanation when we say that the reverse of all these conditions hold when the kidneys are reached. In its transit through the lungs the venous blood loses the carbon dioxide responsible for the increased affinity of its colloids for water. When, now, the arterial blood reaches the kidneys—which, let it be noted, are supplied with extra large arteries—more water is contained in the blood than the blood colloids are capable of holding, and so this separates off as urine.¹⁵ These considerations show why a water absorption is the rule in the intestine and a water secretion the rule in the kidney. How under the influence of an acid the absorption of water by any colloid is

¹³ Hamburger, *Osmotischer Druck und Ionenlehre*, Wiesbaden, 1902, i, p. 291; *ibid.*, p. 404.

¹⁴ These figures are nearly doubled if instead of comparing the sizes of the corpuscles in arterial and in ordinary venous blood the sizes in arterial and passively congested venous blood are compared. In other words, the same circumstances that make the passively congested organ become edematous make the corpuscles in the blood become "edematous."

¹⁵ The question that naturally arises is why the secretion of water does not occur into the lungs (where the CO_2 escapes) instead of through the kidneys. The reason may be twofold. The colloidal membrane between blood and alveolus may not be freely permeable to water (just as the urinary membrane is not in acute parenchymatous nephritis) or the time necessary for a reversal of the water absorption may be greater than the time the blood spends in traversing the lung capillaries.

accomplished constitutes a separate question,¹⁶ as does also that subsidiary one of how the separation of the unbound water through the (colloidal) urinary membrane is effected. (Page 187.)

In place of the teaching of Ludwig that a secretion of urine is primarily dependent upon a blood pressure, or Heidenhain's belief that the velocity with which the blood passes through the kidneys is of primary importance, I venture to suggest, in interpretation of the experiments and clinical observations at hand on this subject, that the normal urinary secretion is absolutely dependent upon an adequate oxygen supply to the cells constituting the parenchyma of the kidney. Any interference with this oxygen supply leads to a decrease in urinary secretion even to the point of absolute and permanent stoppage. Through a particularly favorable oxygen supply to the kidneys the secretion of urine may be increased above that ordinarily considered "normal." (Page 189.)

As the relief of the particularly acute forms of nephritis still presents many clinical difficulties, I have tried to discover if some help might not be obtained from the ideas advanced in this paper. The anuria is probably one of the most striking symptoms of the more intense grades of parenchymatous nephritis that are observed clinically. Such an anuria is obtained in rabbits and dogs with the greatest ease if only the renal artery is clamped for a little while. If the clamp is kept in place even a couple of minutes, a fall in urinary secretion is strikingly apparent for some time afterward, and if left in place long enough, a secretion from the insulted kidney may never again be obtained. The coincident swelling of the kidney in these experiments is promptly relieved by direct injection of various salts (sodium phosphate, sodium sulphate, sodium chloride) either directly into the body of the kidney itself, or into the renal artery or into the general circulation. Most interesting, however, is the fact that a kidney which under ordinary circumstances would never again secrete any urine will do so if these salts are injected. (Page 193.)

How now are these various experimental findings to be interpreted? Let us first call attention to the important experimental error that is introduced into any of these experiments with salt infusion if any anesthetic is used. When enough is used to produce anesthesia, a lack of oxygen in the tissues and a retention of some of the liquid infused may be expected to follow. The effect of an infusion is divisible into

¹⁶ See the theoretical considerations of Wolfgang Pauli and Hans Handovsky, *Biochemische Zeitschrift*, 1909, xviii, p. 353.

two parts: first, the effect of the water injected, second, the effect of the salt. Other things being equal, we may expect the water to behave, so far as diuresis is concerned, just as this behaves when water only is injected. The salt injected has an effect upon the kidney and also upon the colloids of the body generally, including those of the blood and lymph. As the chief salt of the body fluids is sodium chloride, we are not surprised to find that a sodium chloride solution isosmotic with the blood, when injected intravenously, acts about as the injection of an equal amount of water (the minor effects of pure water as a plasmolyzing agent on the blood, etc., not being taken into consideration). If, however, a sodium chloride solution having an osmotic concentration above that of the blood is used, an increased secretion of urine is obtained. This is because the salt acts not only directly upon the colloids of the blood and makes them liberate some of their water, but diffuses into the tissues of the body and makes the colloids here also give up a part of their water. This water is then "free," and can be secreted as urine. The salt also acts upon the colloids of the kidney, making the cells of this organ shrink. This shrinkage of the kidney cells necessarily means a change in the physical constitution of the previously described colloidal membrane that separates the urine from the blood. We can say that this change makes for an "increased permeability" of the membrane, but the real physics that underlies this designation cannot yet be satisfactorily gone into. The higher the concentration of the injected salt the more water must the body tissues yield up for diuresis (and the more "permeable," perhaps, also may we consider the kidney membrane to become to water). (Page 196.)

Frey finds that when water is given a rabbit by mouth or rectum, or is injected intraperitoneally, or into the small intestine, an increased amount of urine is secreted by the kidneys. I would say that this is because the tissues of the rabbit are saturated with water and so none of it is retained. If in place of water a sodium chloride solution is injected, the same or even a greater diuresis is obtained. This diuresis is the greater the higher the concentration of the salt solution injected (the amount of fluid injected being the same), just as in the experiments of my own already described. (Page 198.)

The diuresis following the introduction of water does not occur if any anesthetic is administered (morphine, chloral, ether, urethane). This is evidently because the anesthetics all produce a state of lack of oxygen, so that the tissues have an increased affinity for water and so do not secrete that which has been absorbed from the alimentary tract

or peritoneum. Let Frey's finding be noted that these anesthetics do not interfere with the absorption of water from the gastro-intestinal tract. We are not surprised in the face of our explanation to note that Frey found this retention of water to occur just the same whether he had previously bled the animal or had cut the nerves to the kidneys, or changed the posture of the animal. Not even when he gave phloridzin or salicylic acid in an attempt to "stimulate" the kidneys did he get a urinary flow. According to our ideas of urinary secretion such a result is entirely to be expected. None of these procedures affect the affinity of the colloids of the tissues for water except as some increase it. (Page 198.)

The continuance of an absorption of water from the gastro-intestinal tract while none was being secreted through the kidneys is easily explained by the increased affinity of the colloids of the tissues generally for water. On this basis it is also an easy matter to explain the increased absorption of salt solution from the peritoneal cavity in nephrectomized animals, as observed by S. J. Meltzer and Salant.¹⁷ The retention of substances that should be secreted through the kidneys poisons the tissues and increases the affinity of their colloids for water. (Page 198.)

Let us recall here our division of the urinary secretory system into its three parts: the blood, the secreting membrane, and the urine, and our brief characterization of the first as a liquid colloid in which various crystalloids are dissolved, the second as a solid colloid also containing various crystalloids, and the third as a watery solution of various crystalloids (practically) free from colloids. Thus far our discussion has shown that under the conditions normally existing in the body no water can be introduced into the blood without getting the secretion of an equal amount as urine. And what is secreted as urine is water and only secondarily do substances come to be dissolved in it, so that it assumes a chemical composition which permits it to be characterized as urine. Let us see now what must happen if some soluble¹⁸ (or pseudo-soluble) substance is introduced into the blood. To simplify the problem and not make our discussion unnecessarily long, let us think of the blood as one homogeneous system, and the urinary membrane as another. Under such circumstances one of three possibilities presents itself from a physico-chemical standpoint. The dissolved substance

¹⁷ Meltzer and Salant: *American Medicine*, 1904, viii, p. 194.

¹⁸ The word soluble is used in these paragraphs in its broadest sense, so as to include even the pseudo-soluble (colloidal) substances.

may distribute itself uniformly throughout the blood and the urinary membrane, or it may be present in either a greater or a less concentration in the urinary membrane than in the blood. Just what will happen is dependent upon the nature of the dissolved substance and the physical and chemical composition of the blood and the urinary membrane at the time. Of greatest importance are such facts as the presence and absence of lipoids, the character of the colloids concerned, and the state of these colloids as determined by the presence of acids, alkalies, salts, or various non-electrolytes. In other words, the laws of partition and the laws of adsorption again come into play. These differences in the distribution of a dissolved substance between the blood and the urinary membrane are rendered strikingly apparent when dyes are used as the dissolved substances. (Page 201.)

But this distribution of a dissolved substance between the blood and the urinary membrane represents in the end only a static affair, and the secretion of dissolved substances in the urine is a dynamic one. It requires no special comment to see now why only through the continuous secretion of water from the kidney can a continuous separation of dissolved substance from the urinary membrane (secretion) be rendered possible. The presence of water in Bowman's capsule and in the uriniferous tubules introduces the third phase into our secretory system and breaks down continuously the equilibrium that is trying to become established between the dissolved substance in the blood and the dissolved substance in the urinary membrane. (Page 202.)

The attempt to establish an equilibrium between the dissolved substances in the urinary membrane and the dissolved substances in the urine (originally only water) as it passes down the uriniferous tubules makes for a diffusion of dissolved substances out of the urinary membrane, and so tends to destroy, all the time that water is being secreted by the kidney, the equilibrium which is trying to be established between the dissolved substances in the blood and the dissolved substances in the urinary membrane. When now we recall the physico-chemical fact that when any dissolved substance is offered simultaneously to a liquid colloid, a solid colloid, and water (as is the case in the kidney), an unequal distribution of the dissolved substance between the three phases is the rule, then we will have no difficulty in understanding why a difference in quantitative composition between the blood, kidney tissue, and urine, so far as dissolved substances are concerned, is also the rule. Wherefore a "selective" secretion is to be expected rather than to be wondered at. (Page 202.)

Some of Fischer's "concluding remarks." At the conclusion of his discussion, Fischer makes the following special allusions, among others, to the work of previous observers:

There is no difficulty in understanding why Cohnheim's experiments, in which he combined the infusion of sodium chloride solution with moderate injury to a part, always led to the development of an edema more promptly than the infusion alone. The moderate injury (heat, sunburn, iodine application) simply brought about, by indirect means, the so necessary change in the colloids of the tissues, and the increased affinity for water once being established the water of the sodium chloride infusion quickly satisfied it. (Page 206.)

The interpretation of another experimental observation of Cohnheim¹⁹ seems to me to need revision. Cohnheim found that an animal which has been bled repeatedly, and injected after each bleeding with a sodium chloride solution, finally develops a general edema, and interpreted this as an edema of cachexia, caused through an increased permeability of the bloodvessel walls, determined primarily through a hydremia. Would it not be a simpler interpretation to say that through these frequent bleedings the animal becomes anemic—that is to say, his organs get into a state of lack of oxygen—and when a supply of water is furnished the tissues, whether through a sodium chloride infusion or in any other way, they simply take this up? (Page 206.)

We need not further discuss the inadequacy of the blood pressure theory to account for edema. While Cohnheim regarded blood pressure as one of the two great factors concerned in the production of edema, he also recognized that severe edemas occur in animals when no change whatsoever in blood pressure is apparent. To account for an edema under such circumstances he had recourse to an "increased permeability of the bloodvessel walls." If in the light of our modern physico-chemical conceptions we try to say just what is meant by this, we have to define the bloodvessel wall as a colloidal membrane. From physico-chemical observations we know that the permeability of such colloidal membranes is alterable, so this far Cohnheim is on safe ground. But of what consequence would an increased permeability of the bloodvessels be from a pathological standpoint? To force liquids through the bloodvessel walls is not to force them into the tissues. And the fluid of an edematous tissue is very decidedly in the cells themselves. Cohnheim's hypothesis would simply squeeze the edema fluid

¹⁹ Cohnheim: *Allgemeine Pathologie*, Zweite Auflage, Berlin, 1882, i, p. 498.

as far as the outer wall of the capillaries. If we try to aid Cohnheim's conception of permeability and make it extend to all protoplasm, then we are getting the cause of our edema right where we have tried to say it is, namely, in the tissues themselves; and then our problem is simply that of how tissues hold their water. In this the forces that have been suggested as active—not only the variable affinity of colloids for water, but the previously suggested osmotic pressure, with or without Overton's conception of lipoidal surface layers—are so infinitely greater than the highest grades of blood pressure that pathologists have ever registered that the two cannot be compared. (Page 207.)

The more recent experiments of Magnus have added much to our knowledge of the experimental side of edema. His results, too, are usually interpreted as lending support to Cohnheim's conception of the increased permeability of bloodvessel walls as a factor in the production of edema. How well they support the belief that the cause of edema is to be sought in a change in the colloidal constitution of the tissues is readily evidenced by the following. Magnus found that animals which are transfused after death always develop a general anasarca. Living animals do not do so as readily as the dead, but they do it readily if deeply chloroformed or etherized or injected with arsenic. In place of these words we could write, placed in a condition of lack of oxygen with an adequate supply of water. Magnus also found that animals which have their kidneys removed develop an edema if injected with sodium chloride solution a day or two after the operation. This is because at the end of this time the tissue colloids have either directly or indirectly been so altered by the metabolic products which should have been excreted through the kidneys that their affinity for water has been distinctly increased. (Page 208.)

It is readily apparent that through experimental analysis the part played by the blood and the lymph circulations has gradually become less prominent. From having been looked upon as alone determining the amount of water held by the tissues, we have come to find that the tissues are largely their own masters in this regard. The blood and lymph circulations carry fluid to the tissues and away from them, but what the tissues will take off or give off rests with them. Only as these circulatory systems carry to the tissues substances which directly threaten their existence, or fail to remove such as the tissues have produced, which if allowed to accumulate will overcome them, only in so far are the circulatory systems masters of the tissues. (Page 208.)

Of this possible role of the tissues pathologists have not all been

ignorant, but for the most part their ideas regarding it have been vague. All the more credit, therefore, belongs to Jacques Loeb²⁰ and after him to W. B. Cannon,²¹ who not only first tried to prove through experiment that the problem of edema is essentially a problem of the tissues, but suggested for its explanation a physico-chemical force (osmotic pressure) which if not adequate was at least of such a nature as could be conceived active in the living body. (Page 209.)

Fischer's detailed presentation of his extension of Loeb's theory of edema is concluded with the following paragraph:

What now has been accomplished by this finding that the amount of water held by the tissues is essentially an expression of their colloidal state? Its chief virtue lies in this: It places the problem. When we speak as we have done throughout this paper of the "affinity" of the colloids for water, we have not used this word unthinkingly. An affinity is not a clearly defined force, but we have chosen it to cover a present lack of knowledge concerning the nature of the forces underlying this very important relation existing between a (hydrophilic) emulsion colloid and the water it contains. Physical chemistry has not yet settled for us what this is, but toward the answer to this question it is now striving. When it is obtained we will have to strike out this mysterious word "affinity" and write into its place the names of such clearly defined forces as physical chemistry may choose to dictate. (Page 209.)

The writer's comment on Fischer's work. The foregoing quotations from Fischer's book have been carefully selected to the verge of redundancy in order to afford a clear and complete conception of the collochemical theory of edema as Fischer has formulated it. Fischer has given new force and more pregnant significance to some well known but frequently disregarded facts, and his work will receive sympathetic study wherever colloidal properties are comprehended and appreciated.

²⁰ Loeb: Pflüger's Archiv, 1898, lxxi, p. 468.

²¹ Cannon: American Journal of Physiology, 1902, vi, p. 91. Cannon showed that the cause of increased intracranial pressure after injury is dependent upon changes in the tissues of the brain itself which enable this organ to absorb an increased amount of water. He interpreted his findings as in favor of Loeb's ideas of edema. They are more readily interpretable on the basis of our colloidal conceptions of edema. Had Cannon's results received the recognition which their worth merits, pathologists, clinicians, and surgeons in their discussion of the source of increased intracranial pressure would not still be seeking in blood pressure the origin of a force greater than itself, for the swelling brain is able to shut off its own arterial blood supply.

The intimate and essential relationships which exist between water and the colloids in organisms have never before been so thoroughly considered or so effectively studied. That intracellular hydrophilic coordinations are among the most important functionally in protoplasm has long been evident to all students of cellular and tissue chemistry.²² Fischer's book and papers on this subject give emphasis and impart definiteness to views that have been entertained in many quarters but which have not been clearly or systematically formulated.

Fischer's book is an enthusiastic, earnest, comprehensive, able and interesting presentation of a new and stimulating outlook in a perplexing field. His initiative is bold, his familiarity with collochemistry is intimate, his terms are trenchant, and his pleading is persuasive. His work deserves unstinted praise. His results will receive widespread attention in medical circles. Although his conclusions will arouse opposition, his findings will quicken interest in edema, and will encourage discussion and ultimately will increase greatly our understanding of this very important subject.

Fischer agrees with Loeb in "placing the problem of edema in the tissues." Fischer attributes edema, wherever it occurs, to the collective influence of the organic acids which, in a given case, result locally from *subnormal* oxidation. Dissenting from Loeb's osmotic explanation of the way in which such acid induces local accumulation of water, Fischer emphasizes the power of acid to increase the "affinity of (tissue) colloids for water," and refers the whole of edema to this particular influence.

Lactic acid, in Fischer's view, is the responsible acid in the production of edema. One is surprised, therefore, to find that lactic acid does not figure in the summaries of Fischer's *experimental* data. Fischer's experiments involved treatment of colloids and tissues with large and excessive volumes of stagnant acid solutions. He does not show that sufficient organic acid has ever been produced in a given case of edema to cause directly the water accumulation and the swelling observed. What is the reaction of juice suitably obtained from a typical edematous tissue? Does such liquid contain lactic acid in the free state? Does a fluid of this kind have the

²² Gies: Biochemical Bulletin, 1911, i, p. 68.

power of exciting edema in a normal tissue; or of causing such colloids as fibrin, when immersed in it, to swell; or of absorbing more water than that attracted by an equal volume of similar juice from normal tissue of the same kind? Does the minimal *proportion* of lactic acid which is capable of inducing edema in a living tissue destroy the life of any of the cells or definitely impair normal cellular activity? Does such a proportion of lactic acid remove from its setting in protoplasm any basic factor of importance in the essential intracellular coordinations?

In Fischer's view the water of edema diffuses from the capillaries to the tissue spaces and thence into the cells in response to the attraction exerted for it there by intracellular colloids under the influence of abnormal proportions of acid. Does the production of lactic acid in an edematous tissue keep pace with the inflow and accumulation of water? Does the water which accumulates in a case of edema leave behind it all its former associates in lymph, such as phosphate and bicarbonate, when it enters the edematous field? Does edema result from the action of sodium di-hydrogen (acid) phosphate, or of any other acid salt produced by combinations of intracellular alkalin compounds with any lactic acid that may become available? Does such acid induce edema, or does it combine with or affect protein in any way, *in the presence of an excess of alkalin material?*

Fischer demonstrated the striking power of small proportions of electrolytes as agents which counteract the swelling influence of acids, even when the latter are present in large excesses. He does not explain these effects although he emphasizes their importance and their utility. He shows that tartrate and phosphate anions are particularly active in this respect, but he does not appear to consider the probable tendency of phosphate in the tissues to interfere with the swelling influence of such quantities of lactic acid as may be presumed to occur there under pathological conditions. He fails to indicate how much or how little the lactate arising from neutralizations in such cases antagonizes the bloating influences of the lactic acid which, let us assume, keeps on developing in an edematous tissue.

Fischer shows that *alternate* treatment of colloids with acids

and salins reduces the hydrophilic tendency of the colloids in the presence of acid, but he does not discuss this observation in its relation to similar conditions in cells and tissues.

The diffusion tendencies and effects in edematous tissues are ignored. Does lactic acid accumulate in an edematous tissue as lactic acid or in the form of lactates, or does it pass out of the tissue as acid or as lactates? What is the minimal concentration of lactic acid which is able, in solutions containing the physiological salins in their ordinary proportions, to bring about swelling of any of the colloids or colloidal masses which Fischer used in his experiments? Does this proportion of lactic acid ever occur in edematous tissue? Do *acid* salts favor or retard the tendency of lactic acid to increase the affinity of colloids for water?

Fischer did not consider the probable or possible bearing of definite chemical relationships between the colloids and the acid in the case. Are *definite* unions or relationships between tissue colloids and lactic acid essential to the edematous manifestation of colloidal hydrophilia? If so, do the tissue colloids and lactic acid form a hydrophilic partnership in the presence of such substances as di-sodium hydrogen phosphate and sodium bicarbonate?

Fischer refers to CO_2 as one of the factors in the production of edema but does not show why the large quantities of CO_2 which are normally produced in tissues fail to induce water accumulation in them. Does the intracellular CO_2 have any effect directly or indirectly on the power of *lactic acid* to cause edema?

The experimental procedure in Fischer's work is not always the best that might have been adopted. Fischer's experiments were performed with *dry* masses, and with solid parts of organisms. How his theory could be *experimentally* verified with blood serum or tissue juice (dissolved colloids) in the absence of "membranes" or "partitions" is not suggested. How do *dissolved* colloids, as compared with solid colloids, behave in the presence of lactic acid, and phosphates, and bicarbonates? Do the interstitial *dissolved* colloids have any influence in restraining the development of edema at any stage of the process?

Goodridge and I find, when moist shreds of fibrin are severally suspended in gelatin solution, peptone solution, fresh egg white,

blood, milk, and meat juice, that hydrochloric acid solution (0.2 per cent. to 10 per cent.) may be added to the mixture in each case *in any proportion* without inducing visible effects on the fibrin shreds, *unless sufficient acid is added to provide an excess in the FREE state*. Very large quantities of acid may be added to such mixtures without inducing appreciable bloating effect.²³ If the colloids in the artificial solutions and protoplasmic liquids enumerated above are combined with any proportion of the acid up to exactly their *maximum* affinity for it (hydrochloric acid), so that the liquids while strongly acid to litmus respond negatively to tests for *free* acid, then moist fibrin shreds can remain in such fluids indefinitely without swelling to any perceptible degree. Warm concentrated gelatin solutions may be put into these conditions of free and combined acidity. After such solutions have been permitted to gelatinize, moist fibrin shreds which have been imbedded in the resultant jellies swell perceptibly, provided the gelatinized mass contains *free* acid, *but the shreds do not appear to absorb water from the medium if its contained acid is only in COMBINED form*. It is obvious that such facts have an important bearing on any theory of acid causation of edema.

A few days ago²⁴ I extended these observations to enucleated eyes with similar results and have conducted additional experiments with fibrin and other colloids. After their treatment with 0.05 to 0.2 per cent. hydrochloric acid solution to effect their maximum absorption of water, enucleated eyes and fibrin masses were

²³ Goodridge and Gies: Proceedings of the Society for Experimental Biology and Medicine, 1911, viii, p. 107.

²⁴ "A few days" before the meeting of the Biochemical Association at which this subject was discussed. The results of subsequent experiments will be reported elsewhere.

The writer has lately commented in a very general way on some of these additional results in a preliminary report in the Proceedings of the Biological Section of the American Chemical Society (BIOCHEMICAL BULLETIN, 1911, i, p. 124). The following remark is included in that report: "Experiments with enucleated eyes (from dogs, rabbits and chickens, in solutions of *combined* acids) . . . failed to yield edematous results, but emphasized the need for experiments on *solutions* of biological colloids, such as serum and lymph. Fischer's theory is based upon the results of experiments on *solid* masses in large excesses of acid solutions. He has not shown that his experimental conditions are closely analogous to the natural ones in edema."

immersed in combined acid solutions (Witte peptone in 0.2 to 2.0 per cent. hydrochloric acid solution) where they promptly lost all the water they had previously absorbed from the free acid solution and soon returned to the original dimensions.

All these results suggest that acid would not cause the *gel* proteins in the cells to imbibe water abnormally in the presence of the associated *sol* proteins. The results also warrant the provisional inference that the circulating *sol* proteins would attract (and by osmosis obtain) acid of intracellular origin from any combinations there existing with either intracellular *gel* proteins or the intracellular *sol* proteins, or both. That the circulating *sol* proteins lose to the associated circulating *basic* compounds any acid combined with or adsorbed to the proteins is a justifiable belief. That the salins resulting from such neutralization reduce hydrophilia in the vicinity of their origin and transit before their excretion, is indicated by many observations. "The acid end products of metabolism, without appreciably changing the actual alkaline reaction, constantly take up alkali from blood and protoplasm. In this manner there is a tendency to disturb the normal protective equilibrium between bases and acids. This tendency is held in check by the kidney, which in the process of urine formation reverses the reaction of neutralization of acid and restores to the blood that alkali which has served as a carrier of acid."²⁵

Fischer bases his whole conception on the action of *acid as acid*. That acid, *as acid*, is the responsible and aggressive agent in the production of any *natural* edema is something that I cannot see. On the other hand, that acid by reducing basicity or effecting a reaction-discoordination or inducing some other molecular disequilibrium, may be an *inciting* cause, or a *stimulating* influence, or an *indirect* though none the less influential factor, is quite comprehensible. As a *link* in a *chain* of factors, the influence of acid in effecting abnormal hydrophilia is conceivably important.

Fischer's book has the great merit of sharply stimulating questions. Do any *non-acid products* of intermediary metabolism retard or accelerate the presumed action of lactic acid in edema? Fischer does not discuss this matter. Has it been definitely estab-

²⁵ Henderson: Journal of Biological Chemistry, 1911, ix, p. 423.

lished, directly or by methods of exclusion, that nothing occurs in an edematous part but the production of organic acid to account for an increased affinity of the colloids for water? Has Fischer duly considered, in this connection, the effect of pathocolloidal coordinations in cells, in response to various prevailing influences in incipient edema? Does it follow, because acids increase colloidal hydrophilia and because lactic acid production is increased by suboxidation in tissues as edema is there inaugurated, that the edema is caused or initiated by the resultant lactic acid? Have the possible influences of hydrolases been duly considered? Is it improbable or impossible that such enzymes are even more important factors in the development of edema than the acid which is produced and to which Fischer attributes the whole hydrops? May not enzymes of this kind—aided perhaps by organic acids or acid salts or both—cause such changes in the normal intracellular colloidal coordinations and in the colloids themselves as to result in increasing the total affinity for water by the parts involved?

Are the hydrostatic phenomena of certain edemas clarified by Fischer's conception of directive colloidal hydrophilia? Does his theory account for the great diversity in composition of edematous fluids? Repeated severe hemorrhage, on a free diet, is followed by the urinary excretion of lactic acid in exceptional quantities but there is no visible anasarca.²⁶ Why not? (See page 302.) What is the explanation of the absence of general edema in diabetic "acidosis"?²⁷

Do all observers agree that "the fluid of an edematous tissue is

²⁶ Fischer accepts in general, as I do here in particular, the results of Araki's determinations of lactic acid (lactate), although Araki's studies should be repeated with improved methods. If the special excretion of lactic acid signifies suboxidation, as Fischer assumes, the conditions after several severe hemorrhages without restricted diet appear to be favorable, in Fischer's view, for the causation of edema.

²⁷ If general edema fails to occur in diabetes because of neutralization of the "diabetic acids" or if the acids of diabetic "acidosis" merely neutralize abnormal basicity, in what material respects are these neutralization and inhibitive effects different from those that normally prevail in practically all parts of the living body? Surely, since large proportions of non-electrolytes are practically without inhibitive effects on colloidal attraction for water in the presence of free acid, "diabetic sugar" would not cause the observed difference.

very decidedly in the cells themselves?"²⁸ Is there no excess of interstitial water in edema? Are the *fibrillæ* of an edematous connective tissue bloated? According to Fischer a frog leg immersed in water becomes edematous as a result of postmortem acid formation primarily (autolytic hydrolysis of carbohydrate?) and saline dialysis secondarily (removal of inhibitive factors). But what about the possible effects of *general* autolysis with its consequent protoplasmic discoordinations and preliminary cumulative productions of additional hydrophilic molecules?

Fischer does not touch on the well known effects of the various lymphagogues. This is an interesting and important omission. Twelve years ago Asher and I²⁹ observed prolonged *postmortem* flow of thoracic lymph from a dog. Others have since obtained similar results. What in Fischer's view is the bearing on edema of such phenomena of lymph flow? Does Fischer's theory explain "*edema ex vacuo*"? Are hereditary edemas or neuropathic edemas, natural and experimental, readily explained by this theory?

These are among the questions which require answers before Fischer's theory can be accepted in toto as a complete expression of the whole truth in the matter. From the little I know and understand of the clinical aspects of edema, I fancy clinicians will have numerous questions of their own to add to such as are raised above.

These, and other questions that occur to us, can probably be answered promptly and directly by Fischer from his large experience in the practical studies he has planned and managed so well, but if the answers are presented in his book, I have overlooked them.

That Fischer's statements are not always what he apparently intends them to be is obvious as one reads page after page. It is possible that some doubts in my own mind are due to this fact. Thus he says time after time that this, that, or the other colloid or colloidal mass "swells more in the (!) solution of *any* (?) acid than it does in distilled water." In this case I assume it is his intention to say, in effect, merely that the colloidal mass, in each case, swells more *in each of the particular solutions of the very few acids he has used*, than it does in distilled water. He surely doesn't intend to say

²⁸ Fischer: Loc. cit., p. 207.

²⁹ Asher and Gies: Zeitschrift für Biologie, 1900, xl, p. 207.

in this connection what we find in print. Fischer's readiness in discussion, and his overflowing fullness of the subject, have evidently betrayed him into such errors of presentation.

These criticisms, offered as they are in the interest of further practical development of our knowledge of the subject, happen to be in general harmony with some of Fischer's remarks on his theory that are practically ignored by him after their casual presentation in a few words but which I believe are of more importance than that commonly ascribed to rhetorical alternatives. Let me restate Fischer's most general formulation of his theory:

A state of edema is induced whenever, in the presence of an adequate supply of water, the affinity of the colloids of the tissues for water is increased above that which we are pleased to call normal. The accumulation of acids within the tissues brought about either through their abnormal production, or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible for this increase in the affinity of the colloids for water, *though the possibility of explaining at least some of the increased affinity for water through the production or accumulation of substances which affect the colloids in a way similar to acids or through the conversion of colloids having but little affinity for water into such as have a greater affinity must also be borne in mind.* (Page 99.)

I am convinced that great possibilities of usefulness lie buried in the italicized part of the foregoing quotation. I believe this despite the fact that Fischer practically discards this significant alternative in behalf of his theory of direct acid causation of edema. The following quotations from Fischer's book are far more important, it seems to me, than his "passing" references would indicate:

Under the influence of proteolytic ferments ordinary gelatin can be converted into Beta-gelatin. As already pointed out, Wolfgang Ostwald's studies show this to be capable of greater swelling than the unchanged gelatin. It is therefore conceivable that in inflammation (whether in the eye or elsewhere) an increased affinity of the tissue colloids for water and a consequent edema may result merely in consequence of the "autolytic" changes that occur in the injured tissues, even when no abnormal storage or production of acids in the part occurs. (*Footnote*, page 129.)

The local edemas following the bites or stings of insects have a special interest. In quite a number of these the sting carries formic or other acids into the tissues. Here we have a direct etiological factor for the production of the local edema. In others, poisons are injected which have a well-marked reducing power. By this means a local group of cells are placed in a state of lack of oxygen through chemical means. It is worthy of note that to start with and during the period of greatest swelling such insect stings are white, and not until later do they become red. The increased blood flow so necessary in most explanations of these local edemas does not occur until the edema has begun to subside. Instead of the blood circulation determining the edema, the edema determines whether the circulation shall continue through the affected part or not. (Page 108.)

This explanation of the nature and cause of local edemas can be further tested. The edematous wheals following bites or stings can be mimicked perfectly with a gelatin plate and a little acid. If with a fine hypodermic needle a little formic acid is stabbed into such a gelatin plate, and the whole is then laid into water, an urticarial-like wheal develops about each spot pricked with the needle, which in shape and in the rate of its development is not unlike those which follow the bite of an insect or the introduction of the formic acid laden needle into the skin. (Page 108.)

In Fig. 44 are shown some wheals which developed accidentally on the surface of some of my gelatin discs. The particular disc pictured had lain for thirteen days in a $n/20$ hydrochloric acid solution. The hyaline gelatin does not photograph easily, and so the figure does not indicate how clearly these wheals imitate such as are observed clinically. Those shown here are due to local infections of the gelatin with a mold. In place of the perfectly smooth surfaces such as these gelatin discs ordinarily show, we see them here studded with small mounds indicative of irregularities in the absorption of water. The cause for these local swellings may be a twofold one. As the mold developed while these discs were lying in dilute acid solutions, I question whether an additional local production of acid (of which the molds are capable) gave rise to the local swellings. The affected spots were softer than the surrounding gelatin, and later became almost liquid. I think, in consequence, that the gelatin suffered a partial digestion under the influence of proteolytic ferments manufactured by the mold in the affected spots. Such a partially digested gelatin corresponds with the Beta-gelatin of Traube, and this we know from Wolfgang Ostwald's³⁰ ex-

³⁰ Ostwald: *Archiv für die gesammte Physiologie*, 1905, cix, p. 277.

periments to be capable of a distinctly greater swelling than the ordinary gelatin. (Page 109.)

In passing let it be noted that this simple observation teaches how a chemical change, in this case induced through a ferment, in the colloid itself—just such a change as might occur in living matter—may affect its affinity for water. This is a fact not without biological significance in this problem of the ways and means by which a tissue regulates its water content. (Page 109.)

The last paragraph in the above quotation is among the most pregnant in Fischer's book. If he had gone farther along this line he would have noted previous observations of similar character³¹ and might then have given more attention to the influence of enzymes as possible factors of importance in the development of edemas.

I do not know enough either of collochemistry or of edema to add anything material to the superficial remarks I have already made. Yet it may not be amiss to conclude with the direct suggestion that Fischer's deduction that *acids as acids* are the cause of *abnormal* colloidal hydrophilia in animals does not necessarily follow from any of his information. If his theory of the direct causative influence of acid in the production of edema is sound, it seems to me its correctness still remains to be shown. There is much that looks like analogy and coincidence in Fischer's presentation of the case, but little that resembles positive evidence of *primary* direct acid causation of edema under *biological* conditions.

Perhaps I can emphasize my skeptical view by leading our thoughts into a *non sequitur* similar to the one into which Fischer

³¹ Berg and Gies have found that colloidal water absorption is facilitated by some hydrolytic enzymes (proteases). *Journal of Biological Chemistry*, 1907, ii, pp. 508, 522, and 545. Among our conclusions on this point is the following one (p. 545): "Bloating influences on fibrin were due primarily to the acid or base, but were more pronounced in the presence of enzyme. Elastin did not swell perceptibly in either the acid or basic solutions employed, but did so in the latter when trypsin was present." Similar results were subsequently obtained in preliminary experiments with collagen. Under the head of swelling effects on fibrin we wrote (p. 523): "We intend to repeat the experiments under various conditions and to discuss the significance of the results after more data have been accumulated." This intention has not been executed, although, like many others which have been replaced by more urgent plans, we expect to follow it at an early opportunity.

may have fallen. When starch paste is treated with a moderate excess of very dilute acid solution and the mixture is warmed, certain conditions are instituted which greatly increase the affinity between the colloid and the water, hydration is effected to the point of hydrolytic cleavage, and soluble starch, dextrans and sugars are progressively produced. When saliva is substituted for such an acid solution, similar products are formed, without the aid of heat and in a much shorter period of time. This suggests that salivary digestion is due to acid! Noting, further, that saliva is *acid* to phenolphthalein, suppose we infer that the amylolytic action of saliva is *primarily and directly due to acid secreted into the saliva!* We should then make—as did Fischer—a deduction in accord with some analogous and coincidental experimental findings, but which would not follow from the premises. Would the observations on which our deduction is based be sufficiently exclusive of other possible causative influences to warrant the assumption that salivary digestion, because of these analogies, is due wholly to acidic increase of the affinities for water which are ordinarily shown by the “(hydrophilic) emulsion colloids” under consideration—starches and dextrans? We could not say, from actual knowledge, that such a conclusion would be warranted by existing conditions. We have learned, of course, that the amylolytic action of saliva is primarily and directly due to the amylase in it and not to the acidity which saliva shows toward phenolphthalein, although the prevailing acidity may *promote* the hydrating influence of the salivary amylase, without inducing by itself any hydration that could be detected. We may even concede that ptyalin itself is an acid without being forced to conclude that *acid*, as we ordinarily conceive it, is the primary and direct causative agent in the hydrophilia and hydrolysis observed in the salivary digestion of starch.

Fischer's theory is a very attractive one and has the great merit of simplicity—two reasons for my hope that additional evidence will prove it to be correct in every detail to the further credit of its brilliant author and to the very great satisfaction of every biological chemist.

IV. THE RELATION OF THE HEART AND BLOOD VESSELS
TO EDEMA

William Weinberger

The first experiment made with the view of explaining the nature and cause of cardiac edema was carried out by Richard Lower. It consisted in tying the vena cava inferior and was followed by edema of both legs. These results have since been repeatedly confirmed.

About thirty years ago Ranvier stated that ligation of the femoral vein does not cause edema unless the sciatic nerve is cut, the latter carrying the vasoconstrictors for the blood vessels of the lower extremity. If this nerve is cut, the blood vessels become dilated and the leg receives more blood. Neither venous stagnation per se, nor vasodilation alone, will cause edema. Both factors have to work together to produce it.

Cohnheim made the following experiment: Utilizing the fact that the vasoconstrictors for the vessels of a rabbit's ear are carried by the cervical sympathetic, he cut this nerve on one side, whereupon the corresponding ear became fiery red. He then cut the other sympathetic and ligated the large vein at the root of the corresponding ear. This double operation was followed by edema. The tying of the vein caused an increase of the venous pressure and the cutting of the sympathetic caused an increase of the arterial pressure. The enormously increased intracapillary pressure thus resulting then induced the transudation of such a considerable quantity of lymph that it cannot be carried away by the blood vessels and the lymphatics. Cohnheim's experiments have laid the foundation for many views we entertain to-day upon the pathology of edema. This author, in collaboration with Lichtheim, investigated the question of whether plethora can lead to anasarca, the form of edema which is most characteristic of renal diseases. These investigators found that infusions of large quantities of normal salt solutions into the veins of rabbits and dogs were followed indeed by ascites, edema of the gastrointestinal tissues, and enormous secretions within the gastrointestinal canal. But there was no edema of the skin. In these experiments, then, an enormous hydremic plethora

did not lead to anasarca. In extending their studies, however, Cohnheim and Lichtheim observed that the infusions of normal sodium chlorid solutions would cause an edema of the skin in any part of the body, if the skin was subjected to any moderate irritation, such as a previous exposure to the sun, the painting of this part with iodine or its immersion in hot water for a short period.

As most of these procedures are productive of a mild superficial inflammation—a pathological state characterized by a change in the permeability of the vessel wall—a transudation of a clear lymph rich in proteids results, which is the underlying cause of the so-called inflammatory edema. Cohnheim therefore concluded that the mild irritation of the skin in the experiments mentioned caused a certain alteration of the endothelia of the capillary walls, which made them more permeable to the fluid of the hydremic plethora. However, by repeated transfusions of salt solution, a chronic hydremia is produced, which probably leads to an increase of the permeability of the vessel wall, thus becoming instrumental in bringing out edema of the leg after ligation of the femoral vein.

In this connection mention may be made of the instructive experiments performed by Magnus. Transfusion of blood into an animal, immediately after its death, invariably leads to anasarca. In living animals transfusion leads to edema of the skin, if these animals previously received injections of arsenic. The latter being a specific poison for the blood vessels, its aggravating effect upon anasarca is a strong support of Cohnheim's theory of the importance of the permeability of the capillary wall in the production of edema.

According to Cohnheim the many forms of edema may be reduced to two classes: (1) Edemas which are due to excessive permeability of the capillary wall, and (2) edemas due to venous congestion. Cohnheim lays stress on the fact that even in venous congestion, blood pressure is not the only cause of the resultant edema, the diminished velocity of the blood stream and the increased permeability of the endothelial wall being contributing factors. As to the nature of this altered permeability Cohnheim does not think it due to a simple physical process. He expresses the view that the endothelial cell is a living tissue with a metabolism

of its own. Any change affecting the intracapillary or extracapillary fluid alters the metabolism of the cells, which in turn induces unknown modifications, perhaps of the irritability of the cell.

As instances of edemas due to venous congestion, Cohnheim mentions cardiac edema, and edema due to venous thrombosis. Pure renal edemas—*i. e.*, when without cardiac complications—are due exclusively to an increase of the permeability of the capillary wall, and are therefore to be considered as inflammatory in their origin. Edema in scarlet-fever nephritis is a clear instance of this kind; an inflammation of the skin causes an increased permeability of the walls of the capillaries of the skin. But here, as well as in nephritis, on account of the impaired elimination, the blood contains some toxic agent, which affects the capillary walls. General edemas due to “cold” are caused by irritations of the skin and are, therefore, also of an inflammatory character. The edemas of malaria are brought on by an irritating poison in the blood.

Hydremia, when chronic, gradually changes, as stated before, the permeability of the capillaries. Hence the edemas of anemia, chlorosis and cachexia.

The essential factor in the formation of edema is, according to Cohnheim, the increased transudation from the blood, which can no longer be mastered by the lymphatics. Diminished absorption alone is never the essential cause of edema.

As to the influence of impaired heart action on the genesis of pulmonary edema, Welch's explanation may be mentioned: “A disproportion between the working power of the left ventricle and of the right ventricle of such character that, the resistance remaining the same, the left heart is unable to expel in a unit of time the same quantity of blood as the right heart.” And further: “It is hardly necessary to state that such factors as changes in osmotic pressure, alterations in the capillary endothelium, interference with the absorption of lymph, which have become prominent in the later discussions of the causation of edema, may be utilized in the explanations of pulmonary edema, as of congestive edema elsewhere, but I find great difficulty in conceiving any of these factors alone to be the primary cause of acute general edema of the lungs.”

V. THE BLOOD AS A FACTOR IN EDEMA

Reuben Ottenberg

One of the earliest observations was that of hydremia in nephritis. But later work (by Senator and many others) showed that the hydremia instead of being the cause of the nephritis (as was at first thought), is the sequel of it. In typical edema of chronic nephritis, hydremia only appears after the edema is well established, and is not in any regular proportion to the degree of edema. Furthermore extreme grades of hydremia occur in other diseases (anemia, cachexia), without edema.

One of the most remarkable things about the blood is its great tendency to retain a constant composition. Loeb has shown the importance of constant composition of the surrounding medium for the lower organisms. For the more delicate adjustments of the higher organisms a still greater constancy in the composition of the fluids which connect the cells seems to be necessary. Injections of all sorts of substances—water, salts, proteins, into the circulation, are followed by prompt removal of the substances from the circulation, either by excretion in the kidney, or by absorption in the tissues.

The theories which connect certain types of edema with salt retention are in agreement with this knowledge. The old empirical observation of the therapeutic value of the milk diet led Widal, Strauss, and others to investigate the peculiarity in this diet which gave the good results. They found that it was the low salt content; that by addition of salt to the milk it could be robbed of its virtues, and conversely that other forms of salt-poor food gave equally good, and sometimes better results. Widal divides nephritis into two types—the type with diminished power of sodium chloride excretion, and consequent tendency to edema, and the type with diminished power of excretion of the products of nitrogen metabolism and the tendency to uremia. In the former type the value of the salt-poor diet has been confirmed by many observers. Others have shown that not only chlorides, but also other salts, such as phosphates and carbonates, are of importance. Widal also made the important observations that the appearance of external edema is

only an expression of a water retention which usually begins long before the occurrence of external edema. He believes, of course, that when the kidneys are unable to excrete salt, water has to be retained with the salt to keep the tissue fluids as nearly constant in composition as possible.

From the great variety of clinical cases of edema, it is obvious, however, that this is only one of many causes of edema. Many of the other causes have nothing to do with the composition of the body fluids and have no effect on the blood. Thus, in the edema due to stasis (whether local, as in injuries of veins and lymphatics) or general, one finds only such blood changes as are secondary to the stasis.

VI. RENAL EDEMA

Herman O. Mosenthal

The dropsy which occurs as a symptom of kidney disease presents certain characteristics which differentiate it to some extent from other varieties of edema. There is puffiness about the eyelids and face on waking in the morning; during the day, while the patient is in an upright position, the ankles swell and this swelling gradually creeps upward toward the knees. The genital organs are frequently involved early in the disease. It is evident that some of these edematous accumulations are influenced by gravity while others are not. That mechanical factors are not sufficient to account for them is further emphasized by the fact that the edema is occasionally localized in atypical situations, as for instance the chest wall, the arms or the pleural cavity.

The fluid spreads from these areas, involving the entire skin and subcutaneous tissues. This condition is spoken of as *anasarca*. Transudation into the peritoneal, pleural and pericardial cavities occurs only at a later period. The order of these events must be borne in mind since in experimentally produced edema the *anasarca* frequently appears last instead of first or not at all.

Attempts have been made to trace an etiological relationship between the glomeruli or the tubules of the kidney and edema. On the supposition that the former excrete the fluid portions of the urine while the latter are mainly responsible for the elimination of

the solid constituents, a lesion of either one of these renal elements might be followed by edema. To establish such a sequence of cause and effect would be very interesting. However, there are many obstacles to be overcome before it may be considered proved. In the first place it is almost impossible in either acute or chronic nephritis to find kidneys which present involvement of only one portion of the renal structure. In the second place no conclusion as to the physiological efficiency of the parts may be obtained by microscopic inspection, nor is it possible, as the clinician knows, to foretell the pathological condition of the kidney from a study of the patient.

This is the position in which histological pathology has left the question. It must however be realized that even if portions of the kidney appear diseased they may still be able to function normally; and conversely, symptoms of disease may be accompanied by no apparent histological change. Shlayer, Hedinger and Takayasu have recently emphasized this independence of function and structure. In experimental nephritis it has been found that the uranium and chromate salts produce kidney lesions which are identical. However, while edema and oliguria occur with the former this is not the case with the latter. Testing out these two forms of nephritis, these authors found that in spite of the similar appearance of the kidneys under the microscope, great diversity in the excretory functions exists. That histological pathology has almost yielded its utmost contribution to such problems as that of renal edema is generally appreciated and investigators are turning more and more to experimental pathology and physiology for their solution.

Although it is impossible to associate definite kidney lesions with the occurrence of edema, there is a constant relationship between it and renal disease caused by certain poisons. Scarlet fever, pregnancy, streptococcus infections often of the tonsil and also elsewhere, diphtheria and other diseases and conditions frequently give rise to nephritis with edema. Exactly similar lesions of the kidneys occur from other causes but are not accompanied by this symptom. The same is true of the experimental types of nephritis. It is tempting under such conditions to assume that the edema does not

depend on the nephritis but that both edema and nephritis are the effect of a common cause. Conditions in scarlet fever particularly seem to point to such a relationship, for here signs of edema may precede those of the nephritis and a very marked edema may occur without symptoms of a kidney lesion.

Richard Bright early in the nineteenth century attributed renal edema to a watery condition of the blood, a hydremia, brought about by loss of albumin through the urine. It is now known that in every case of renal edema the albumin content of the blood is diminished and its specific gravity lessened, that is, that the hydremia postulated by Bright actually exists. The diluted blood was supposed to pass through the capillaries more readily than the normal blood serum. Later studies have shown this to be improbable. It was found that not all cases of kidney disease exhibiting albuminuria suffered with edema, and furthermore in conditions where a loss of albumin through other channels occurred no edema could be detected. Hence hydremia per se, unassociated with other abnormal states, is discredited as the cause of renal edema.

The dropsy accompanying nephritis might on first thought be considered capable of a simpler interpretation. Since the kidney is the main outlet for the waste fluid of the body, the loss of its power to excrete fluids would result in marked accumulation of water. This retention of water would in the first place dilute the blood, that is produce a "hydremia," and in the second place, wherein this hydremia differs essentially from that brought about by loss of albumin, increase the total volume of the blood and thus result in a condition of plethora.

The relation of hydremic plethora to edema may be tested experimentally in two ways: Either by diminishing the urinary flow or by increasing the volume of the blood by infusion.

The effect of diminished urinary flow has been closely observed in animals after ligation of both ureters and after nephrectomy, and in human beings after the accidental simultaneous closure of both ureters by urinary calculi or new growths. These subjects continuing their normal solid and fluid intake retain all the waste products ordinarily secreted in the urine and yet develop no edema.

It is possible that not enough fluid is retained in such compara-

tively short periods of absolute anuria to produce a grade of hydremic plethora comparable to that obtaining in renal dropsy.

By the infusion of normal saline solution an extreme condition of hydremic plethora may be produced. Cohnheim and Lichtheim in classical experiments infused rabbits and dogs with large quantities of fluid. They thereby produced ascites, edema of the gastrointestinal tract and other organs, but the edema of the skin and subcutaneous tissues, the anasarca characteristic of renal disease was lacking. Fluid had not passed through the skin capillaries into the interstitial spaces. It was therefore deemed probable that besides plethora and hydremia, a lesion of the capillary blood vessels is necessary to bring about an anasarca.

The blood vessels of the skin may be injured by the application of iodine, exposure to the sun and other means. Infusion in animals which have been thus treated results in local edema corresponding in extent to the application of the irritant. Furthermore if a blood vessel poison, such as arsenic, is injected, infusion will result in anasarca comparable to that seen in nephritis.

Therefore the production of renal edema may be assumed to be brought about by no single cause but by a chain of events: Hydremic plethora associated with an injury of the smaller blood vessels and a kidney lesion. The order in which these occur makes no difference, so far as is known. However that all three must be present has recently been shown by Pearce in experiments on rabbits. Injecting a variety of blood vessel and renal poisons and producing hydremic plethora by giving water through the stomach tube, this investigator proved conclusively that not any one or any two of these factors were sufficient to bring about anasarca and dropsy of the body cavities, but that all three must cooperate to produce edema.

In this explanation of renal dropsy the problem of the influence exerted by the kidney itself on the condition is especially hazy. As was pointed out above, neither hydremic plethora nor a blood vessel lesion will in itself produce edema. The retention of sodium chloride and other substances normally excreted in the urine bears on the problem only in so far as these substances influence fluid retention and increase the hydremic plethora. These points have

been discussed at greater length in one of the previous papers. What the exact rôle of the kidney lesion in the symptom complex is is not known. That the renal involvement is an indispensable factor was surmised some time ago and has been further demonstrated by the experiments of Pearce and others. According to Senator the same poison which damages the blood vessels also affects the kidney. The structure of this organ, characterized by a double set of capillaries closely interwoven with the glomeruli and tubules, lends probability to this theory. On the other hand H. Strauss would reverse the process and claims that the blood vessels become diseased because of the retention of urinary products following the onset of nephritis. This theory is also within the limits of possibility.

A generation ago renal edema was thought to be due to the combined action of hydremic plethora, a blood vessel lesion and a diseased kidney. Recent experiments have strengthened and amplified this idea essentially but have not succeeded in elucidating it completely.

THE BITE OF RUSSELL'S VIPER¹

A. E. SPAAR

(Civil Hospital, Trincomalee, Ceylon.)

At midnight on April 6, 1910, I was hastily summoned to see the late Mr. MacIntyre, Postmaster of Trincomalee, who had been bitten by a polonga. On arrival at his residence, thirty to forty minutes after the accident, I found him seated erect on a chair on his verandah. He was bathed in a cold, clammy sweat, and complained of feeling sick, and was vomiting continually. The ejected matter consisted of a few grains of boiled rice and water and bile-stained fluid, and later on of glairy mucus. He had been attended to, within five or ten minutes of the accident, by a constable, who applied to the wound a black "snake stone" such as I have seen in the possession of "snake charmers." Internally a remedy, prepared by dissolving part of a light green stone in water, had been administered with the object of producing vomiting.

The Postmaster stated that about ten minutes after he retired to bed he heard a noise as of heavy breathing, and imagining that it was his little boy who was asleep, he walked over to the latter's cot, about four feet away. Making him comfortable, he was returning to his own bed, when he felt a sharp sting over his heel, and jumped into bed. Simultaneously, hissing sounds were heard, and it immediately struck him that he must have been bitten by a snake. A light was brought into the room, which had been in darkness, and a search made, and a polonga was found coiled up in a corner. Three hemp ligatures were applied by his wife round the injured limb: one just above the ankle, another round the knee, and the other round the lower part of the thigh. The wound is said to have bled freely, staining all the bed linen. Careful examination, after cleansing of the limb, revealed a single, black, pin-point puncture on

¹ Reprinted, through the courtesy of Dr. Louis Hussakof, from *Spolia Zeylanica*, 1910, vi, pp. 188-190.

the inner side of the right heel, about an inch above the sole. There was then no bleeding, and very slight pain. The surrounding tissues had a faint bluish tint, and the limb was swollen from the knee downwards. The ligatures, I found, were not too tightly applied. The patient complained of great weakness, and there was much restlessness, violent retching, and inability to sleep.

I incised the wound freely, injected into it a saturated solution of permanganate of potash, and also made a series of punctures all round it. The same solution was injected hypodermically into the tissues. Powdered crystals were then rubbed in, and the wound packed with the same. The limb was postured, and compresses of the solution also applied and frequently renewed.

Four fluid ounces of whisky and half an ounce of sal volatile were administered internally at once, and a full dose of strychnine and ether injected hypodermically into the arms an hour later. The subsequent treatment consisted of a mixture of carbonate of ammonium, citrate of caffeine, strychnine, and digitalis, and hypodermic injections of adrenalin and strychnine. The treatment adopted was that described by Dr. J. W. Watson Stephens, and in his hands proved very successful in Siam. The vomiting ceased after the first dose of whisky had been administered. I was not certain as to whether the vomiting and cold sweats were due to the snake poison or to the emetic administered by the constable, but it was evident later that these were effects of the former. The poison, therefore, had undoubtedly entered the general circulation before I first saw the patient. At dawn the patient was not so restless, but complained of great thirst and hunger. The bowels had acted once and were relaxed, the skin was warm, the tongue dry, the expression anxious, and the eyelids had now a very heavy appearance, the patient being unable to open them wide. The elevators of the lids exhibited parietic symptoms. The pupils were contracted, fixed, and equal. Pulse was quick, 115 per minute, and moderately full.

Finding that the ligatures were rather lax, I proceeded to remove them, following the procedure recommended by Prentiss Willson in the *Archives of Internal Medicine*, June, 1908, by intermittingly relaxing the ligature nearest to the heart, letting it become looser

and looser until it was entirely removed. The other ligatures were removed in the same manner, the effect on the patient being noted at the same time. At midday vomiting commenced again, but was not persistent. The tissues all about the wound were slightly tumefied and inflamed. Bleeding took place every now and again, especially if the patient exerted himself. A noteworthy feature of the blood was that it was thick, dark in color, and did not coagulate. Restlessness was more marked. Weakness, depression, and exhaustion and pains in the small of the back were complained of, but there were no cramps, no paralysis of the limbs, and no convulsions. The skin again began to break out in cold, clammy sweat. The abdomen was distended and tympanitic, the upper part exhibiting a board-like hardness. Eructations were frequent, but did not appear to relieve the patient. He complained of suffocating pains, as if both sides of his chest were being compressed. There was great oppression. Respiration was hurried and labored, and the pulse was becoming weak and more rapid—125 per minute. Sight was rather dimmed, but recognition of objects and persons was possible. Sinapisms were applied to the feet and over the præcordial region. Saline infusions were injected per rectum. The patient seemed to rally somewhat, the pulse falling to 118 per minute. At this stage, however, his case was taken over by a native "snake physician of known repute," and English treatment given up, but the case was watched by me with interest to the end.

Drops were instilled into the eyes by the "vedarala," and this appeared rather to aggravate the dimness of sight. Internal remedies were also administered, but with the withdrawal of stimulants there was a steady rise in the pulse, till at 5 P.M. it registered 132 beats per minute, and was soft and feeble. Respiration also became more hurried and difficult.

At 10 P.M. the pulse rose to 142 per minute, and slight signs of lividity were noticed about the face. The native physicians were now making preparations against the twenty-fourth hour, which is stated to be a critical time with cases of snake bite. At about 11 P.M. dried bile from chickens was insufflated into the nostrils, which made the patient feel very short of breath. Within a couple

of minutes he called to his wife to hurry quickly to him, and taking leave of her dropped back on his pillow and expired instantly. Consciousness and the power of speech were retained to the very last. Death appeared to have been due to asphyxia and heart failure, and *I am firmly convinced that free stimulation from the very onset is strongly indicated in cases of snake bite, if only to prevent the extreme exhaustion which marks these cases.*

The external appearances noticed eight hours after death were lividity of the face, which was almost black. The lower portion of the face was swollen. Livid patches were also seen on the neck, chest, and lower extremities. The palmar aspect of the fingers was black in color, and the nails were of a deep purple hue. A blood-stained fluid was issuing from the mouth and nostrils. The pupils were widely dilated, and the eyeballs congested. Post-mortem rigidity had disappeared, and decomposition set in early.

ABSTRACTS OF BIOCHEMICAL LITERATURE

Department of Biological Chemistry in Chemical Abstracts

WILLIAM J. GIES

With the publication of the first number of *Chemical Abstracts* in October, the writer succeeded Professor Lafayette B. Mendel in the editorship of the Department of Biological Chemistry.¹ A plan for the classification of the biochemical abstracts was inaugurated in the issue of October 20. The classification is intended to facilitate access to all the abstracts pertaining to any general biochemical subject in a given issue. The plan has been growing in effectiveness with each succeeding number of *Chemical Abstracts*. In the issue of December 10 the biochemical abstracts were grouped under the following heads: *General, methods and apparatus, bacteriology, botany, physiology, pathology, pharmacology, zoology*.

Dr. C. F. Langworthy, editor of the Department of Nutrition in *Chemical Abstracts*, having long desired to resign that editorship, retired on October 31. Dr. Langworthy had been the editor of the Department of Nutrition from its establishment and had made the department one of the most serviceable in *Chemical Abstracts*. The writer succeeded Dr. Langworthy and has been classifying the nutrition abstracts in each issue in two sections: *Normal and abnormal*.

Beginning with the first issue in January, 1912, the former Departments of Biological Chemistry, Immunochemistry, and Nutrition, in *Chemical Abstracts*, will be merged in an enlarged Department of Biological Chemistry under the writer's general editorship. This merger will effect a very gratifying coordination of all the essentially biochemical abstracts in each issue. The abstracts will be grouped in sections, under the supervision of assistant editors. There will also be a comparatively large addition to the number of abstractors.²

¹ BIOCHEMICAL BULLETIN, 1911, i, pp. 141 and 160.

² The abstractors in each of the three departments under the old regime will continue in practically every case under the new. The names of the abstractors will be published in the March issue of the BIOCHEMICAL BULLETIN.

In the consummation of this plan the writer will have the coöperation of the colleagues named in the appended list of sections and sectional editors:

- A. **General**—PROF. FRANK P. UNDERHILL, *Yale University*.
- B. **Methods and apparatus**—PROF. STANLEY R. BENEDICT, *Cornell University Medical College*.
- C. **Bacteriology**—DR. ERNEST D. CLARK, *New York Botanical Garden*.
- D. **Botany**—DR. CARL L. ALSBERG, *Bureau of Plant Industry, U. S. Department of Agriculture*.
- E. **Nutrition (Normal and Abnormal)**—PROF. P. B. HAWK, *University of Illinois*.
- F. **Physiology**—PROF. ANDREW HUNTER, *Cornell University*.
- G. **Pathology**³—PROF. H. GIDEON WELLS, *University of Chicago*.
- H. **Pharmacology**—PROF. ALFRED N. RICHARDS, *University of Pennsylvania*.
- I. **Zoology**—DR. ROSS A. GORTNER, *Carnegie Institution's Station for Experimental Evolution*.

Professor Mendel created the Department of Biological Chemistry in *Chemical Abstracts* five years ago and, with the earnest cooperation of an able corps of abstractors, made the department a great success in every respect. We hope the enlarged Department of Biological Chemistry will fully meet the expectations of all who use *Chemical Abstracts*. We have accepted our responsibilities in this work in the conviction that the prompt preparation and presentation, in English, of satisfactory abstracts of the entire biochemical literature is a professional and public function of great general usefulness. We hope that every biological chemist will be a "constant reader" of the biochemical abstracts, and that all biologists will continually avail themselves of the opportunity to obtain from *Chemical Abstracts* the gist of the great mass of biochemical information which is presented on its many pages and which is essential in the development of biological investigation.

³ The former Department of Immunochemistry, edited by Professor Wells, will be merged, with Professor Wells' approval and coöperation, into the Section of Pathology.

Volume 5 of *Chemical Abstracts* (1911) contains 4,000 pages of abstracts, with a complete and detailed index of subjects and names of authors (about 1,000 pages additional). *Chemical Abstracts* is issued bi-monthly, on the 10th and 20th. The subscription price, to non-members of the American Chemical Society, is \$6.00 per annum. Remittances, made payable to Professor Charles L. Parsons (Secretary of the American Chemical Society) and addressed to the BIOCHEMICAL BULLETIN, will be forwarded to headquarters.⁴

⁴Unavoidable delay in the publication of this number of the BIOCHEMICAL BULLETIN enables us to add the following information regarding the first three issues of *Chemical Abstracts* in 1912:

January 10. Department of Biological Chemistry—22 pages, 148 abstracts.

January 20. Department of Biological Chemistry—23 pages, 126 abstracts.

February 10. Department of Biological Chemistry—26 pages, 134 abstracts.

All the plans referred to above have been successfully consummated.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

PROCEEDINGS REPORTED BY THE SECRETARY,

WALTER H. EDDY

I. SECOND REGULAR SCIENTIFIC MEETING (SECOND ANNUAL BUSINESS MEETING)¹

The second regular scientific session of the Association, as well as the second annual business meeting, was held on June 5, 1911, at the College of Physicians and Surgeons. In the absence of the President, Dr. Nellis B. Foster, the chair was occupied by Dr. Gies. About thirty members were present. The scientific program consisted of a symposium on edema, as follows:

- I. Lymph and its relation to edema.....William J. Gies.
- II. The earlier theories of edema.....Jacob Rosenbloom.
- III. Fischer's theory of edema.....F. G. Goodridge.²
- IV. The relation of the heart and blood vessels to edema.
Wm. Weinberger.
- V. The blood as a factor in edema.....Reuben Ottenberg.
- VI. Renal edema.....Herman O. Mosenthal.

Abstracts of the papers are published in this issue of the *BIOCHEMICAL BULLETIN* (pp. 270-324).

After a recess an executive session was held, the following business being transacted after the reading and approval of the minutes of the first annual meeting.

Biochemical Bulletin. Dr. Gies outlined plans for the publication of the *BIOCHEMICAL BULLETIN*. The proposed plans were approved.

¹The proceedings of previous meetings are given on pages 57-93 of the *BIOCHEMICAL BULLETIN*, 1911, i.

²Dr. Goodridge was unavoidably prevented from attending the meeting. In his absence Fischer's theory of edema was discussed by Dr. Gies.

Officers. Officers were elected to serve for the year 1911-1912, as follows:

HONORARY OFFICERS. *Honorary President:* Alfred N. Richards, Professor of Pharmacology at the University of Pennsylvania, Secretary of the American Society of Biological Chemists, and Editor of the Journal of Biological Chemistry.

Honorary Vice-Presidents:

P. B. Hawk, Professor of Physiological Chemistry at the University of Illinois, and author of "Practical Physiological Chemistry."

William N. Berg, Biological Chemist, Bureau of Animal Industry, U. S. Department of Agriculture.

Carl L. Alsberg, Chemical Biologist, Bureau of Plant Industry, U. S. Department of Agriculture.

Max W. Morse, Professor of Biology, Trinity College.

Ross A. Gortner, Biological Chemist, Carnegie Institution's Station for Experimental Evolution (Cold Spring Harbor, L. I.).

ACTIVE OFFICERS. *President:* Herman O. Mosenthal; *Vice-President:* Jacob Rosenbloom; *Secretary:* Walter H. Eddy; *Treasurer:* William J. Gies.

Additional members of the Executive Committee: William H. Welker, Nellis B. Foster, Alfred P. Lothrop.

Additional members of the Editorial Committee: William H. Welker, Nellis B. Foster, Jacob Rosenbloom, Alfred P. Lothrop, Emily C. Seaman, Ernest D. Clark, Reuben Ottenberg, Anton R. Rose, Clayton S. Smith, Edgar G. Miller, Jr.

Members elected. In accordance with Article II, Section I, of the Constitution,³ the gentlemen named below were unanimously elected to membership:

Herman M. Adler, Instructor in Psychiatry, Harvard Medical School.

Albert H. Allen, Practicing Physician, Saranac Lake, N. Y.

Hugh Auchincloss, Department of Surgery, Columbia University.

Louis Baumann, Assistant Professor of Medicine, University Hospital, Iowa City, Ia.

Chas. F. Bolduan, Bacteriologist, Department of Health, New York City.

Edward M. Colie, Jr., Surgeon, Out Patient Department, Bellevue Hospital, New York City.

³ BIOCHEMICAL BULLETIN, 1911, i, p. 58.

- William Darrach, Instructor in Surgery, Columbia University.
Donald Gordon, Instructor in Physiology, Columbia University.
Alfred F. Hess, Investigator, Board of Health, New York City.
Frederick B. Humphries, Pathologist, German Hospital, New York City.
Peter Irving, Assistant in Clinical Pathology, Columbia University.
Clinton B. Knapp, Physician, General Memorial Hospital, New York City.
Chas. C. Lieb, Instructor in Pharmacology, Columbia University.
Wm. H. McCastline, Assistant Professor of Physical Education, Teachers College, Columbia University.
James P. McKelvy, Physician, Pittsburgh, Pa.
B. S. Oppenheimer, Fellow in Pathology, Columbia University.
Alwin M. Pappenheimer, Associate in Pathology, Columbia University.
Edwards A. Park, Assistant in Medicine, Columbia University.
Maximilian Schulman, Instructor in Applied Therapeutics, Columbia University.
Hermann von W. Schulte, Assistant Professor of Anatomy, Columbia University.
Leander H. Shearer, Instructor in Physiology and Medicine, Columbia University.
Edward Anthony Spitzka, Professor of General Anatomy, Jefferson Medical College.
Ralph G. Stillman, Assistant Clinical Pathologist, New York Hospital, New York City.
Wm. K. Terriberry, Instructor in Physiology, Columbia University.
F. T. Van Beuren, Instructor in Surgery, Columbia University.
Wilbur Ward, Associate in Gynecology, Columbia University.
Herbert B. Wilcox, Assistant in the Department of Diseases of Children, Columbia University.
William H. Woglom, Pathologist, St. Luke's Hospital, New York City.

Before adjournment refreshments were served and a social meeting enjoyed.

II. FIRST ANNUAL DINNER

The initial meeting of the Biochemical Association during the present academic year was made the occasion of the first annual dinner of the Association. To the surprise and delight of all concerned, one hundred and twenty members and guests assembled for

the dinner, at the Hotel St. Denis on December 13. The committee of arrangements had been privileged to announce to the members that Prof. R. H. Chittenden, the first director of the Biochemical Department, would honor the association by his presence and that he would make an informal after-dinner address. As the guest of honor, Professor Chittenden was informally welcomed before the dinner by the members of the association in the parlors of the Hotel St. Denis. The association was favored, also, by the presence with Professor Chittenden of other distinguished guests, among them Dr. James P. Atkinson, Prof. S. P. Beebe, Dr. Ralph C. Benedict, Dr. F. J. Birchard, Dr. Martha Doan, Dr. D. W. Fetterolf, Dr. Morris S. Fine, Dr. Samuel C. Harvey, Dr. Warren H. Hilditch, Dr. J. Morgan Howe, Dr. Holmes C. Jackson, Dr. Walter A. Jacobs, Dr. F. B. La Forge, Prof. Frederic S. Lee, Dr. P. A. Levene, Dr. Jacob G. Lipman, Dr. Jacques Loeb, Prof. John Marshall, Prof. Frank S. Meara, Dr. Francis H. McCrudden, Dr. S. J. Meltzer, Dr. Victor C. Meyers, Dr. Herman Schwarz, Dr. Donald D. Van Slyke, Prof. Francis C. Wood. The members of the Association found it a great pleasure to greet informally so many eminent investigators under auspices that were at once so happy and inspiring. Drs. Winifred J. Robinson and William N. Berg came from Poughkeepsie and Washington, respectively, to join their fellow members on this occasion.

As the dinner progressed and the psychical stimulations increased in number and intensity with the infective manifestations of good will on every side, "*physiological economy in nutrition*" was ruthlessly ignored—even by Professor Chittenden himself!

At a comparatively late hour after a prolonged period of good fellowship, the President of the Association, Dr. Herman O. Mosen-thal, felicitated the Association on the success which thus far had characterized all its affairs and which had culminated in so splendid a gathering for such an amiable purpose. The President read a letter from Dean Lambert, of the Medical School, in which the Dean, after congratulating the Association on its success as an organization and on its promise of a useful career, expressed his regret that he could not participate in the dinner and take an active

part in the professional and personal tribute to his old teacher and friend, Professor Chittenden. Dr. Mosenthal concluded his opening address by introducing Professor Gies as toastmaster.

Professor Gies, in response to the President's remarks, expressed his appreciation of the personal compliments which the President had bestowed. He spoke of the scientific significance of the presence of so many women. He indicated his pleasure to greet so numerous a body of his past and present coworkers. He drew attention to the fact that he was addressing the largest assembly ever convened in America under the professional banner of biological chemistry.

Professor Gies appropriately introduced each of his friends and colleagues on the appended program.

Introductory Remarks. The development of biochemical instruction and research in two centers of medical education.

In Philadelphia. JOHN MARSHALL, *University of Pennsylvania*.

In New York. JOHN A. MANDEL, *New York University and Bellevue Hospital Medical College*.

Professor Chittenden's relation to the establishment of the Columbia University Department of Biological Chemistry. FREDERIC S. LEE, *Columbia University*.

A Columbia medical student's impressions of Professor Chittenden as a teacher. EDWARD A. SPITZKA, *Jefferson Medical College*.

Professor Chittenden as a director of research. P. A. LEVENE, *Rockefeller Institute for Medical Research*.

Professor Chittenden's influence on the advancement of medical research. FRANK S. MEARA, *Cornell University Medical College*.

Informal Address. Reminiscences of earlier days in physiological chemistry. RUSSELL H. CHITTENDEN, *Director of the Sheffield Scientific School and Professor of Physiological Chemistry, Yale University*.

The program of the evening evolved an interesting exposition of the historical development of biological chemistry in America. Combined with this review were many tributes of honor to and respect for the man so largely responsible for the development of biochemical science in this country—our guest of honor, Professor Chittenden.

Professor Marshall gave a most interesting and illuminating outline of the early days of biological chemistry, beginning with Wöhler's classic preparation of urea. Professor Mandel was unable to be present but sent a very interesting historical paper which was read by his colleague, Prof. H. C. Jackson. Of direct interest to the members of the Association were Professor Lee's reminiscent remarks on the establishment of our own Biochemical Department and on the relation of Professor Chittenden to its foundation. In the enforced absence of Professor Spitzka, President Mosenthal read a letter from him that added another to the tributes to Professor Chittenden which this occasion coördinated in a very happy manner. Dr. Levene analyzed for us in a most impressive and delightful way Professor Chittenden's contribution to the spirit of American research. Professor Meara concluded the toasts with humanly appreciative touches on the career, influence and personality of our guest of honor and his old teacher.

Before formally presenting Professor Chittenden, Dr. Gies spoke earnestly and appreciatively of his long period of intimate association with Professor Chittenden, as pupil and assistant, at Yale and Columbia. He referred to the Biochemical Association as one of the remote but none the less obvious effects of Professor Chittenden's influence. He attributed much of the present success of the Biochemical Department at Columbia to Professor Chittenden's continuing spirit with us. Dr. Gies introduced Professor Chittenden as his "old master and friend, the Dean of American Biological Chemists."

Professor Chittenden then responded feelingly to the expressions of esteem to which he had been forced to listen silently up to this time, and in a brief and intimately informal manner gave a review of his early experiences as student, teacher and investigator in physiological chemistry.

At the conclusion of the program Professor Gies proposed the election of Professor Chittenden as the first honorary member of the Biochemical Association. The motion to this effect was adopted unanimously by a rising vote and with hearty applause.

Before adjournment Professor Gies, with very evident pleasure,

congratulated the members of the association on the brilliant success of the dinner and expressed the hope that an unbroken chain of similar annual events might grace the future history of the Biochemical Association.

The names of the members and guests at the dinner, and the groupings at the tables, are indicated below :

The Guest of Honor—RUSSELL H. CHITTENDEN.

The President—HERMAN O. MOSENTHAL.

The Toastmaster—WILLIAM J. GIES.

Guests of the Association

James P. Atkinson	P. A. Levene	Francis H. McCrudden
S. P. Beebe	Jacob G. Lipman	Frank S. Meara
†Robert A. Hatcher	Jacques Loeb	S. J. Meltzer
Holmes C. Jackson	†John A. Mandel	†Edward A. Spitzka
†S. W. Lambert	John Marshall	F. C. Wood
Frederic S. Lee		

Members and Their Guests

William N. Berg	Chas. F. Bolduan	Wm. B. Boyd
*D. W. Fetterolf	Edward M. Colie, Jr.	Walter H. Eddy
Donald Gordon	Frederic G. Goodridge	†Nellis B. Foster
*Walter A. Jacobs	*Rolfe Kingsley	*Wm. H. Gilson
Gustave M. Meyer	Jacob Rosenbloom	Clinton B. Knapp
†Archibald E. Olpp	*Herman Schwarz	Chester A. Mathewson
*Donald D. Van Slyke	†Ralph G. Stillman	Raymond C. Osburn
†William Weinberger	W. K. Terriberly	Reuben Ottenberg
William H. Welker	F. T. Van Beuren, Jr.	Chas. H. Vosburgh
	†J. W. Weinstein	
*Martha Doan	*Jennie R. Bear	Blanche R. Harris
Mabel C. Little	*Lillie H. Dahlgren	Louise McDanell
*Marie L. Minor	*Harriet C. Jacobson	Jessie A. Moore
Winifred J. Robinson	*Emma Jeffery	Blanche E. Shaffer
Emily C. Seaman	*Sarah L. Lewis	Mary E. Sweeney
Helen S. Watt	Helen McClure	Ethel W. Wickwire

* Guest of a member of the Biochemical Association.

† Detained or obliged to leave before the conclusion of the dinner.

*F. J. Birchard	Anna E. Everson	Donald B. Armstrong
J. J. Bronfenbrenner	Helen Gavin	Elmer W. Baker
Isidor Greenwald	Beatrice Helen Gross	*Morris S. Fine
*Samuel C. Harvey	Tula Lake Harkey	*Warren H. Hilditch
*F. B. La Forge	Ellen Beers McGowan	*Victor C. Myers
Daniel R. Lucas	Sadie B. Vanderbilt	L. A. Robinson
Hermann J. Muller		Anton R. Rose
	*Willis P. Baker	†Oscar M. Schloss
†T. A. Erpf-Lefkovich	*Douglas M. Dold	J. Buren Sidbury
*Egmont Koenig	*Julius Hyman	Clayton S. Smith
Shojiro Kubushiro	*Geo. G. McElvare	
Percy W. Punnett	*Alan DeF. Smith	
David F. Renshaw	*James A. Steel	
Edward C. Stone	*Vincent Tanzola	*Ralph C. Benedict
T. F. X. Sullivan		*Bertram T. Burton
Grover Tracy	Louis E. Bisch	Ernest D. Clark
	Melvin G. Herzfeld	*Dayton J. Edwards
David Alperin	Louis Hussakof	Harry L. Fisher
B. G. Feinberg	Max Kahn	A. J. Goldfarb
Samuel Gitlow	†Darwin O. Lyon	Edward G. Griffin
J. L. Kantor	Edgar G. Miller, Jr.	E. Newton Harvey
*George W. Keil	*Clayton E. Royce	*J. Morgan Howe
*John P. Petty	Charles Weisman	Alfred P. Lothrop
Leo L. Roth		Louis Elsberg Wise
Geo. M. Street		Harold E. Woodward

The complete success of the dinner was due in very large measure to the efficient service of the committee of arrangements: Professor Wm. H. Welker, *Chairman*, and Drs. Alfred P. Lothrop and Ernest D. Clark.

WALTER H. EDDY, *Secretary*.

* Guest of a member of the Biochemical Association.

† Detained or obliged to leave before the conclusion of the dinner.

BIOCHEMICAL NEWS, NOTES AND COMMENT¹

I. GENERAL

Necrology. Dr. F. Beute, director for many years of the control station for fertilizers, feeding stuffs, foods and seeds at Ebstorf, Prussia.

Dr. Antoine Blatin, former professor of physiology at the Ecole de medicine de Clermont.

Mr. Charles Emil Dohme, formerly vice-president of the firm of Sharp & Dohme, sometime president of the American Pharmaceutical Society and president of the Maryland College of Pharmacy.

Dr. Max Jaffe, professor of pharmacology at the University of Königsberg.

Dr. Arthur H. Koelker, biological chemist in the research laboratory of the Roosevelt Hospital.

Prof. Oskar Kellner, director of the Agricultural Experiment Station at Möckern.

Surgeon General Walter Wyman, of the U. S. Public Health and Marine Hospital Service.

Honors. Prof. Svante Arrhenius, of Stockholm, has been elected an honorary member of the Academy of Sciences in Vienna.

The Royal Society has awarded a Royal medal to Dr. W. M. Bayliss, F.R.S., for his distinguished achievements in physiological research.

The Nobel prizes for 1911 in the sciences have been awarded to Mme. Marie Curie, of the University of Paris, in chemistry; to Prof. Wilhelm Wien, of the University of Würzburg, in physics,

¹It is intended to make the sections of "*biochemical news, notes and comment*" in the BULLETIN a series of items of historic value and personal interest to biological chemists. The notes comprising the section in this issue have been compiled at random, but succeeding numbers of the BULLETIN will present more systematic compilations of this character. The cooperation of all our colleagues in the execution of this plan is cordially invited.

and to Prof. Allvar Gullstrand, of the University of Upsala, in medicine.

Prof. Paul Ehrlich, of Frankfort, has been awarded the Liebig medal by the Verein Deutscher Chemiker.

Dr. Emil Fischer, of Berlin, has been awarded the Berzelius medal of the Swedish Medical Society. The Belgian Academy of Sciences has elected as foreign members Professors Emil Fischer and J. Pawlow, of St. Petersburg.

Dr. Simon Flexner, director of the Rockefeller Institute for Medical Research, has been awarded the Cameron Prize in practical therapeutics by the University of Edinburgh in recognition of his work in cerebrospinal meningitis. In accordance with custom Dr. Flexner has been invited to deliver an address at the University of Edinburgh. Dr. Flexner has received from the German government an appointment as honorary member of the Institute for Experimental Therapy at Frankfort-on-the-Main.

The colleagues, friends and pupils of Prof. Armand Gautier, professor of chemistry in the Medical Faculty of the University of Paris and president of the Academy of Sciences, on November 26 celebrated the fiftieth anniversary of his connection with the university.

Dr. Emil Godlewski, professor of agricultural chemistry of the University of Krakau, Poland, has been elected a corresponding member of the Paris Academy of Sciences.

Dr. Jacques Loeb, of the Rockefeller Institute for Medical Research, has been elected a member of the Academy of Sciences in Krakau, Austria.

Dr. S. J. Meltzer, head of the department of physiology and pharmacology at the Rockefeller Institute for Medical Research, is the fifth American to be elected a member of the Imperial Leopoldina Carolina Academy of Naturalists.

The first reception of the Medical Club of Philadelphia for the year was given at the Bellevue-Stratford Hotel, October 6, in honor of the recently elected Professors in the University of Pennsylvania—among the latter, Profs. A. N. Richards and Alonzo E. Taylor.

At a meeting of the Philadelphia Alumni Society of the Medical Department of the University of Pennsylvania, on November 18th, Professor Taylor gave an account of how physiological chemistry is taught and Professor Richards detailed the methods employed in teaching pharmacology.

Dr. E. A. Schäfer, professor of physiology at Edinburgh, has been elected president of the British Association, for the meeting to be held next year at Dundee. Professor Schäfer has been elected a member of the Imperial Academy of Sciences at Halle. The University of St. Andrews has conferred its doctorate of laws upon Dr. Schäfer and upon Sir J. J. Thompson, professor of physics at Cambridge.

The degree of LL.D. was conferred upon Dr. Harvey W. Wiley, Chief of the Bureau of Chemistry, by the University of Vermont on the occasion of the installation of President G. P. Benton.

Appointments. Prof. H. V. Arney, dean of the Cleveland School of Pharmacy of Western Reserve University, has succeeded Prof. Virgil Coblentz at the N. Y. College of Pharmacy.

Dr. William H. Brown has been appointed plant physiologist at the Bureau of Science, Manila, P. I. Dr. R. P. Hibbard, of the Mississippi Agricultural Experiment Station, is his successor at the Michigan Agricultural College.

Dr. W. A. Cannon, of the Desert Laboratory, is acting director of the Department of Botanical Research of the Carnegie Institution during the absence of Dr. D. T. MacDougal, who is traveling and studying desert conditions in Upper Egypt and portions of the Soudan.

E. P. Cathcart, M.D., D.Sc., Grieve lecturer in chemical physiology at the University of Glasgow, has been appointed research associate of the Carnegie Institution of Washington, and is spending the present academic year in research on metabolism in the Carnegie Nutrition Laboratory in Boston.

Dr. M. T. Cook has resigned as plant pathologist in the Delaware Agricultural Experiment Station to become professor of plant pathology in Rutgers College and plant pathologist in the New Jersey College Station.

Dr. Daniel W. Fetterolf has resigned as demonstrator of chemistry and toxicology in the University of Pennsylvania, to accept the position of acting assistant surgeon in the U. S. Army, with a permanent station in New York. Dr. Fetterolf has been commissioned by the U. S. Senate to take charge of the Chemical Department of the U. S. Army Medical Supply Department in New York City.

Dr. E. C. Franklin, professor of organic chemistry at Stanford University since 1903, has been appointed professor of chemistry in the Hygienic Laboratory of the U. S. Marine Hospital Service.

Mr. J. B. Hanson has been appointed instructor in physiology and pharmacology at the University of Colorado.

Dr. Theodore C. Janeway has been elected a member of the Board of Scientific Directors of the Rockefeller Institute for Medical Research, to succeed Dr. C. A. Herter, deceased. He has also been elected President of the American Society for the Advancement of Clinical Investigation for 1911-12.

Professor König has retired as director of the agricultural experiment station at Münster, after 40 years of service, and has been succeeded by Prof. A. Mörner, formerly vice-director.

Prof. J. G. Lipman has been made director of the experiment station and of the college farm at Rutgers College.

Mr. L. F. Shackell has been appointed instructor in pharmacology in the St. Louis University School of Medicine.

Prof. H. C. Sherman is serving on the general committee for Agricultural Chemistry of the International Congress of Applied Chemistry, the National Commission on Milk Standards, the New York Milk Committee, and as reviewer of the chemistry of food and nutrition for the American Year Book. Professor Sherman was recently appointed a member of the administrative board of instruction and research of the newly organized School of Agriculture at Columbia University.

Mr. A. B. Stout, of the University of Wisconsin, has been appointed director of the laboratories at the New York Botanical Garden.

The Directors of the Otho S. A. Sprague Memorial Institute of Chicago will devote the income of the fund chiefly to medical research. Prof. H. Gideon Wells will direct the work, which will be conducted in coöperation with the following existing institutions: University of Chicago, Rush Medical College, Presbyterian Hospital and the Children's Memorial Hospital, of Chicago. The Advisory Council consists of Dr. Frank Billings, Prof. E. R. LeCount, Prof. Ludvig Hektoen, Dr. James B. Herrick, Prof. Edwin O. Jordan, Dr. Joseph Miller and Prof. Julius Steiglitz. Professor Wells has already appointed the following members of the research staff: Dr. R. T. Woodyatt, Dr. Evarts Graham, Dr. H. F. Helmholz, Dr. H. J. Corper, Dr. Lydia Dewitt, Miss Maud Slye and Dr. Wilber Post. Several fellowships will provide for investigations of medical problems.

A food and drug laboratory has been organized in connection with the department of chemistry at the Montana State College at Bozeman. The Montana State legislature at its last session passed a pure food law in which provisions were made for the equipment and maintenance of this laboratory. Messrs. W. M. Cobleigh, state chemist, C. E. Millet, director of drug analyses, Drury L. Weatherhead, food analyst and D. B. Swingle, bacteriologist, constitute the laboratory staff.

Lectures and addresses. Ether day was observed October 16 at the Massachusetts General Hospital in Boston by the usual clinics and luncheon. In the afternoon Dr. Simon Flexner gave an address on "The biological basis of specific therapy." The alumni met at a banquet in the evening and were addressed by Drs. Simon Flexner, Charles F. Stokes, Surgeon-General of the U. S. Army, and Harvey Cushing.

At the first meeting of the year of the Biological Club at the Oregon Agricultural Collège, Prof. Victor L. Gardner gave an address on "Fundamental factors of plant nutrition with special reference to the blueberry." A second address on "The relation of plant pathology to the other biological sciences" was given by H. L. Rees, of the crop-pest staff.

The annual Herter lectures were delivered at the Johns Hopkins

University on October 4, 5, and 6, by Prof. Albrecht Kossel, of the University of Heidelberg, who was awarded the Nobel prize last year for his discoveries in biological chemistry. The subject of the lectures was "Protein metabolism."

Five of the first six Harvey lectures of the "Seventh Course" at the New York Academy of Medicine related to matters of biochemical interest. The names of the lecturers, with the subjects and dates of the lectures, are appended:

October 7. Local specific therapy of infections. Dr. Simon Flexner.

October 14. Ueber den chemischen Bau der Zelle. Prof. Albrecht Kossel.

October 28. Narcosis. Prof. Max Verworn.

November 25. Illuminating gas and the public health. Prof. W. T. Sedgwick.

December 16. A consideration of the nature of hunger. Prof. Walter B. Cannon.

Dr. Paul Lindner, of the Institute for Fermentation Industries at Berlin, gave an illustrated lecture on "New views on fermentation and the fermentation organisms" at the College of the City of New York on Tuesday, October 24, and at Columbia University on Wednesday, October 25.

"The rôle of the salts in the preservation of life" was the subject of the Wesley M. Carpenter lecture, delivered at the New York Academy of Medicine on October 19, by Dr. Jacques Loeb, of the Rockefeller Institute for Medical Research.

At the meeting of the National Academy of Sciences, held in the Public Library, New York City, on November 21 and 22, biochemical papers were presented by Dr. Jacques Loeb on "Oxidations in the cell," and by Dr. T. B. Osborne and Prof. Lafayette B. Mendel, on "The rôle of different proteins in nutrition and growth."

The Silliman lectures for 1911, ten in number, were devoted to the subject of "Irritability" and were given daily at Yale University, beginning on October 9, by Prof. Max Verworn, of the University of Bonn. Professor Verworn lectured at Columbia University on October 26, on "Life and Death."

Among the speakers at a meeting of the Pure Food and Drug Department of the National Civic Federation, held in the rooms of the N. Y. Board of Trade and Transportation on October 2, were Dr. Harvey W. Wiley, Chief of the Bureau of Chemistry; Dr. Thomas Darlington, ex-Commissioner of Health for the city of New York, and William C. Woodward, secretary of the American Public Health Association.

In memoriam. *Ellen H. Richards.* The October number of the *Journal of Home Economics* was issued as a memorial of the late Mrs. Ellen H. Richards. Mrs. Richards's leadership in the Home Economics Movement is clearly portrayed and many personal tributes are presented.

Confident that it is a duty and a privilege to make permanent the inspiration and influence of a life marked to an unusual degree by sanity, wisdom and helpfulness, the American Home Economics Association proposes to raise a fund of \$100,000 in memory of its organizer and former president, Mrs. Ellen H. Richards. The income from the fund—which will be invested and administered by a responsible Board of Trustees representing Mrs. Richards's family and The American Home Economics Association—is to be expended in putting on a firm foundation the *Journal of Home Economics*, the official organ of the Association, and in scholarships and prizes to encourage research work on problems relating to home life. In order that the gift may be a thoroughly democratic expression of appreciation and enthusiasm, and that it may be participated in by all of Mrs. Richards's friends and by everyone interested to contribute toward the cause of right living, the money will be collected in one hundred thousand one-dollar subscriptions. Every cent will go toward the fund, as a few of Mrs. Richards's personal friends will meet all the expenses connected with collecting the money. Contributions may be sent to any member of the committee: Dr. C. F. Langworthy, Office of Experiment Stations, Washington, D. C.; Miss Isabel Hyams, 26 Wales St., Dorchester, Mass.; Miss Ednah A. Rich, 303 Soto St., Santa Barbara, Cal.; Dr. B. R. Andrews, Teachers College, N. Y. City; Mrs. William H. Barrett, Chairman, 108 Johnson St., Brooklyn, N. Y.

John Morgan. The *Journal of the American Medical Association* states that a committee consisting of Provost Edgar Fahs Smith, Ph.D., Dr. S. Weir Mitchell, Sir William Osler and Drs. William Pepper, Clarence Payne Franklin and Swithin Chandler has been formed to take up the project of erecting, in Philadelphia, a fitting monument to John Morgan, founder of the first medical school in the United States, and director-general of the hospitals and physician-in-chief of the American army during the Revolutionary War.

Christian A. Herter. In response to an invitation issued by the President of the Johns Hopkins University and the Committee on the Herter Memorial Lectureship, a meeting in memory of the late Dr. Christian Archibald Herter was held in the lecture room of the Physiological Laboratory on the afternoon of October 5. Drs. W. H. Welch, H. S. Halsted, J. J. Abel, E. K. Dunham and Simon Flexner spoke of various aspects of the life and work of Dr. Herter and paid tribute to his character and his services to medical science.

Endowments. The British Association for the Advancement of Science has recently made an appropriation of \$150 for the furtherance of chemical study of plant enzymes.

The million dollar fund for the further endowment of the Medical School of Western Reserve University has been completed.

The English government has voted \$250,000 for agricultural research, including plant and animal physiology, pathology, breeding and agricultural zoölogy, and fruit breeding. This appropriation is accompanied by a yearly sum of \$15,000 for special investigation. The plan includes grants to various educational institutions (a separate subject to be treated by each institution receiving aid) for investigation and scientific advice to farmers.

Mr. Andrew Carnegie has given \$25,000,000 to the Carnegie Corporation of New York, which was incorporated by the legislature last June. The objects of the corporation are "receiving and maintaining a fund or funds and applying the income thereof to promote the advancement and diffusion of knowledge and understanding among the people of the United States, by aiding technical

schools, institutions of higher learning, libraries, scientific research, hero funds, useful publications, and by such other means as shall from time to time be found appropriate therefor."

Miscellaneous. Radium Institutes. The new Radium Institute was officially opened in London last August. It possesses radium salts to the value of about \$250,000.

At Joachimsthal, a Bohemian mining place, the government has erected an institute which will be devoted entirely to the use of radio-active substances for medical purposes. It is well known that the classical discoveries of Becquerel and Curie, in Paris, were made on uranium salts obtained from material found in the mines of Joachimsthal. Soon afterward exhaustive researches on the mother substance in Joachimsthal showed that the water in the mines is the most radio-active natural water in the world. A private syndicate was soon organized for the purpose of exploiting this natural resource; but public opinion forced the government to assume control of the affair and to erect a *Curanstalt*, a therapeutic institution, accessible to all classes of the population. From the material obtained in these government mines—formerly they yielded a fair amount of silver, too—a large quantity of radium bromid can be manufactured, and in the institute all kinds of treatment by radio-active substances will be given. The building was recently opened; it contains radioactive baths, a large quantity of radium salts, and will be open all the year round. By pipes the water is drawn into the baths directly from the bottom of the mines, and filtered in such a way that it does not lose anything of its activity. The institution is in charge of an expert medical radiologist from the University of Vienna.

New buildings. Six new buildings will be erected at the University of Wisconsin during the present academic year. The erection of a new building for the department of agricultural chemistry, to cost approximately \$100,000, has been started.

For the past two years Roosevelt Hospital has had a research laboratory under an endowment given by Mrs. E. H. Harriman. Recently Dr. Lewis R. Morris offered to erect a suitable building for laboratory work and such a building will be erected in the center

of the Roosevelt Hospital premises. Dr. William Gordon Lyle will have charge of the new institution.

University medical clinic. Prof. Theodore C. Janeway has initiated the organization of a University Medical Clinic at the Presbyterian Hospital with Professors W. T. Longcope and David Bovaird, and Drs. Herbert S. Carter and Herman O. Mosenthal as the other members of the division staff.

Institute for chemical research. An institution for furthering the progress of scientific chemistry without the obligation of teaching will be founded at Dahlem near Berlin. The institute will be erected jointly by the Kaiser Wilhelm-Gesellschaft für wissenschaftliche Forschungen, by a society founded especially for this purpose consisting principally of proprietors of chemical factories, and the state of Prussia. The society guarantees a yearly contribution of \$15,000 and for the building alone \$225,000. The government will give the ground and promises to supply one of the professors of the university as the director of the institution. The management of the new imperial institute will be in the hands of a committee.

Wellcome historical exhibition. Mr. Henry S. Wellcome is organizing and will direct an historical exhibition of rare and curious objects relating to medicine, chemistry, pharmacy and the allied sciences, at the same time as the meeting of the International Medical Congress, which will be held in London in 1913. For many years Mr. Wellcome has been engaged in researches respecting the early methods employed in the healing art, both among civilized and uncivilized peoples. It has been his object in particular to trace the origin of the use of remedial agents, and inquire why and how certain substances came to be employed in the treatment of disease. The exhibition will be *strictly professional and scientific in character*, and will not be open to the general public. Mr. Wellcome would value any information sent him in regard to medical lore, early traditions or references to ancient medical treatment in manuscripts, printed works, etc. Mr. Wellcome desires ultimately to publish, in collected form, all the information obtained. Communications may be addressed to Mr. Henry S. Wellcome, at Snow Hill Buildings, London, E. C.

The Méker burner. The everyday tools of the chemist determine to a large extent the character of his work no less than those of the artisan do his. The success or failure of a great research problem in chemistry may depend as much upon the apparatus at hand as upon the imagination and skill of the worker. Possibly the leading fact in the history of science is this: that great trains of discoveries have depended more upon the invention of new apparatus than upon the development of the human brain. After all it is the attention to the details of equipment as well as the personal organization in a laboratory which brings about perfect results. And so we sing the praises of the Méker burner. With this burner one can do almost the work of the blast lamp, without the annoyance connected with the use of the latter. The flame is large and intensely hot and the highest temperature, strange to say, is reached at the base of the flame. For analytical work in crucibles it has no equal, nor is there any other device approaching it in excellence. It has no inner cone and platinum ware can be made to receive the full effect of the flame without danger of injury. "There is nothing new in the apparatus—no original idea involved," many a critic would say, "just a Bunsen burner with an abundant air supply and a piece of Davy safety-lamp gauze at the top." But not every burner so constructed will give a flame free from the destructive inner cone, and intensely hot at the base. Every point in the design must be carefully balanced to produce the perfect result. We do not know the inventor or the history of his invention, but a simple inspection of the burner in action indicates that this is no chance discovery, no day-dream or night-dream suddenly made concrete and perfect. Carefully thought out and wrought out by trial and experiment and repeated experiment is the perfectly simple Méker burner.—*W. D. Richardson.*

A new paint-destroying fungus. Mr. George Massee in the *Bulletin of Miscellaneous Information of the Royal Botanic Gardens*, Kew, England (No. 8 of this year), describes a new fungus (*Phoma pigmentivora* Mass.) which grows on fresh paint. The fungus grows best in hothouses, high temperatures and constant humidity being especially conducive to its development.

The fungus appears as numerous, small, rose-colored specks in

the white paint about a month after it has been applied. These spots increase in size and change to a purple or dark-red color suggesting the idea of blood having been sprinkled on the paint. The discolored areas spread and finally form effused patches several inches in diameter. The fruit of the fungus appears as minute blackish-red warts. One firm of painters during the present year lost over \$1,000 in consequence of the appearance of the fungus in a large number of cucumber-houses painted with expensive protective paint.

The spores germinate in pure linseed oil but the mycelium remains colorless and produces no fruit. No germination takes place when the spores are sown in pure white lead. The red color suggests that the white carbonate of lead undergoes some chemical change induced by the presence of the fungus and resulting in the formation of oxid of lead. The presence of 2 per cent. of carbolic acid in paint completely arrests the development of the fungus.

This is another illustration of the growth of certain fungi under conditions which would naturally be thought to be toxic to any living plant.—*F. J. Seaver.*

II. DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, OF THE UNIVERSITY OF ILLINOIS

Professor Hawk was recently elected Chairman of the University of Illinois Section of the American Chemical Society. He is now engaged in preparing the manuscript for a *fourth* edition of his "Practical Physiological Chemistry."

Dr. Paul E. Howe, instructor in the department, spent a portion of the summer at the University of Chicago doing advanced work in physiology. Though elected to the position of professor of physiology and physiological chemistry at the Birmingham Medical College, Dr. Howe has accepted an increase of salary and reappointment at Illinois. Dr. Howe represented the Gamma Alpha graduate scientific fraternity at the annual meeting in Washington during the holidays.

Dr. H. A. Mattill, who took his Ph.D. degree in this department in 1910, was recently made associate professor of physiology and physiological chemistry at the University of Utah.

Mr. C. C. Fowler ('09) has been made assistant professor of chemistry at Iowa State College.

Mr. D. W. Wilson, who spent the year of 1910-11 in graduate work at Illinois, is now engaged in cancer research at the New York Hospital under the direction of Prof. Stanley R. Benedict.

Mr. F. Wills ('10) holds an important position in the employ of Armour and Company. Mr. L. A. Fritze ('11) is very satisfactorily located in Pittsburg where he holds a position in the Water Works Department. Mr. N. R. Blatherwick (graduate student, 1910-11) is located at Sioux City, Iowa.

Plans have been drawn for a new building to house the various chemical activities of the University. The portion devoted to physiological chemistry will be very thoroughly equipped with all forms of apparatus essential to investigation in the various lines of research in physiological chemistry. Particular emphasis will be placed upon graduate instruction.

Graduate instruction was given to several candidates for graduate degrees during the summer session of 1911. It is planned to include undergraduate instruction during the 1912 summer session. As in former years, a few students from the household science department are taking work in physiological chemistry. Eleven men are now engaged in research work in physiological chemistry and of these, eight will take advanced degrees in the spring.

The workers in the laboratory have made the following contributions to journals since the end of the last academic year:

P. B. Hawk. Post-anesthetic glycosuria;¹ Urine formation during ether anesthesia;¹ The activity of the pancreatic function under the influence of copious and moderate water drinking with meals;¹ and A modification of Wohlgemuth's method for the quantitative study of the activity of the pancreatic function.¹

Paul E. Howe, H. A. Mattill and P. B. Hawk. The influence of an excessive water ingestion on a dog after a prolonged fast.²

H. A. Mattill and P. B. Hawk. A method for the quantitative

¹ Archives of Internal Medicine (July-October, 1911).

² Journal of Biological Chemistry (December, 1911).

determination of the fecal bacteria;³ The utilization of ingested fat under the influence of copious and moderate water drinking with meals;⁴ The distribution of bacterial and other forms of fecal nitrogen and the utilization of ingested protein under the influence of copious and moderate water drinking with meals;⁴ and Fecal output and its carbohydrate content under the influence of copious and moderate water drinking with meals.⁴

S. R. Wreath and P. B. Hawk. On the allantoin and purine excretion of fasting dogs.⁴

The following papers were reported at the annual meetings of the American Chemical Society, The American Society of Biological Chemists and the American Physiological Society in Baltimore and Washington during the holidays:

Paul E. Howe and P. B. Hawk. A comparison of the data from two fasts, each exceeding one hundred days in length and on the same subject; On the hydrogen ion concentration of feces; and A metabolism study on a fasting man.

D. W. Wilson, Paul E. Howe and P. B. Hawk. The distribution of urinary nitrogen as influenced by the ingestion of moderate and copious quantities of distilled water at meal time.

D. W. Wilson and P. B. Hawk. On the relationship between water ingestion and the ammonia, phosphate, chloride and acid concentration of the urine.

Lawrence T. Fairhall and P. B. Hawk. The fecal amylase output during fasting and water drinking, and The allantoin output of man as influenced by water ingestion.

N. R. Blatherwick, C. P. Sherwin and P. B. Hawk. Intestinal putrefaction and bacterial development accompanying water drinking and fasting.

E. L. Ross and P. B. Hawk. Further studies on the metabolic relationships of ether anesthesia.

³ Journal of Experimental Medicine (October, 1911).

⁴ Journal of the American Chemical Society (October or December, 1911).

III. COLUMBIA BIOCHEMICAL ASSOCIATION

In memoriam. At the annual meeting of the American Society of Biological Chemists, in Baltimore, December 27, Professor Gies presented the following memorandum, on the death of Dr. Pond, which was adopted unanimously by a rising vote:

"The American Society of Biological Chemists records with deep regret the death of Dr. Raymond H. Pond. Dr. Pond was one of the charter members of the Society, and an active and gifted biochemical investigator. Dr. Pond's untimely death removes a lovable and engaging personality from our scientific circle, and a vigorous force in botanico-chemical research has been lost to American science."

Appointments and honors. Messrs. Edgar Altenberg and C. A. Schwarze have been appointed assistants in botany at Columbia University.

Dr. C. W. Ballard was chairman of the committee in charge of the arrangements for the annual banquet of the Alumni Association of the College of Pharmacy, held on December 6. Dr. Ballard was recently elected a member of the Torrey Botanical Club.

Dr. L. E. Bisch, who has been an interne at the Manhattan State Hospital, since last June, was recently given charge of a service of over 500 patients in acute and chronic psychoses. He will deliver several public lectures during the winter on the nature of mental diseases.

Prof. R. Burton-Opitz, and Drs. C. Stuart Gager, Louis Husakof and Gustave M. Meyer have been elected members of the Radium Institute of America.

Drs. Herbert S. Carter and Herman O. Mosenthal have been appointed assistant visiting physicians to the Presbyterian Hospital.

The Association of Official Agricultural Chemists has appointed Dr. A. D. Emmett a referee on methods for the separation of the nitrogenous substances of meats.

Among the five national officers of Phi Lambda Upsilon are Dr. A. D. Emmett, *Vice-President*, Dr. George D. Beal, *Registrar*, and Dr. George A. Geiger, *Treasurer*.

Mr. Harry L. Fisher was recently appointed instructor in chemistry at the Cornell University Medical College.

Dr. George A. Geiger is now chemist to the American Viscose Co., Marcus Hook, Pa.

Dr. A. J. Goldfarb has been made an instructor in natural history at the College of the City of New York.

Dr. A. H. Kropff is now president of the Hoffman and Kropff Chemical Co.

Mr. Darwin O. Lyon is a University Fellow in Psychology at Columbia.

Dr. Clarence E. May has been elected vice-president of the Indiana chapter of Sigma Xi.

Dr. A. McD. McAfee is chief chemist of the Texas Oil Company at Bayonne, N. J.

Dr. B. S. Oppenheimer is Alumni Association Fellow in Pathology at Columbia Medical School.

Dr. Edwards A. Park has been appointed pathologist to the New York Foundling Hospital.

Mr. Fred J. Seaver has been appointed curator of fungi at the New York Botanical Garden.

Dr. Matthew Steel has been promoted to an assistant professorship of physiological chemistry at the University of Missouri.

Miscellaneous. The proceedings of the second annual meeting and the first annual dinner of the Biochemical Association are published on pages 332-339.

Abstracts of the symposium on edema at the June meeting of the Biochemical Association are given on pages 270-324.

The next regular meeting of the Biochemical Association will be held about the middle of March. Prof. Wilder D. Bancroft has accepted an invitation to address the Association on "*The study of environment.*"

Dr. Gertrude S. Burlingham spent the summer near West Wardsboro, Vermont, continuing her study of the genus *Russula* for the North American Flora.

Cerium oxalate is on the list of articles dropped from the last edition of the U. S. Pharmacopœia. The recent study of the effects

of cerium oxalate by Drs. George Baehr and Harry Wessler under Dr. Gies' direction made it evident that cerium oxalate can have little or no special use as a therapeutic agent.

Dr. C. Stuart Gager delivered a lecture, at the New York Botanical Garden on September 9, on the subject of "Plants and People of Pinar del Rio, Cuba."

Drs. Ross A. Gortner and B. S. Oppenheimer were recently elected to membership in the Society for Experimental Biology and Medicine.

Dr. F. M. Hanes spent the summer in Munich studying pathological chemistry.

Dr. Michael Heidelberger writes from Zürich that he is deriving a great deal of benefit from research under Professor Wilstätter's direction. "Professor Wilstätter's remarkable personality, his clear-headed suggestions and his brilliant methods and conceptions are truly inspiring." Before going to Zürich, Dr. Heidelberger inspected the chemical laboratories in many of the university towns in Belgium, Holland, France and Germany, and enjoyed delightful interviews with Professor Franchimont in his magnificent laboratories for organic chemistry at Leyden and with Professor Anschütz at Bonn.

Dr. Alfred M. Hellman recently addressed a meeting of the Anesthetists of New York City and vicinity on "The Anesthetic situation in New York."

Dr. H. D. House, of the Biltmore Forest School, has done considerable collecting the past season in Michigan and Oregon. Fleishy fungi have been scarce, but a number of interesting woody forms have been found and studied in relation to their hosts. Dr. House remarks in a recent letter from Oregon that "except for *Ganoderma oregonense* and *Echinodontium tinctorium*, the woody fungi and wood-destroying fungi do not appear to differ much from those in the east, the same species being common."

Prof. Wm. H. McCastline has been appointed a member of the faculty of the new School of Practical Arts at Teachers College, Columbia University. (See page 361.)

Prof. Raymond C. Osburn delivered, at Trinity College on December 15, a lecture on "Fishes."

Miss Winifred J. Robinson, of Vassar College, and Mr. Benjamin C. Gruenberg, of the Brooklyn Commercial High School, recently passed public examinations for the Ph.D. degree at Columbia University. Biological chemistry was one of the two minor subjects in the advanced courses of each candidate.

Prof. William Salant, Chief of the Pharmacological Laboratory of the Bureau of Chemistry, is organizing and directing the equipment of a laboratory for experimental pharmacology in the Medical Department of Georgetown University. Heretofore this institution has never given instruction in pharmacology. Prof. Salant is creating the Department of Pharmacology in that University.

The Baugh Institute of Anatomy of the Jefferson Medical College, erected at a cost of \$125,000, by Mr. Daniel Baugh, was dedicated on September 26. Prof. Edward A. Spitzka delivered one of the dedicatory addresses.

The names of the following members of the Biochemical Association appear on the official programs of national scientific societies in session in Baltimore and Washington during the holidays:

H. M. Adler	William J. Gies	H. J. Muller
Carl L. Alsberg	Ross A. Gortner	Anton R. Rose
George D. Beal	William T. Horne	Jacob Rosenbloom
S. R. Benedict	P. B. Hawk	William Salant
Isabel Bevier	Michael Heidelberger	Charles H. Sanford
Samuel Bookman	Max Kahn	Emily C. Seaman
Ernest D. Clark	A. H. Kropff	Fred. J. Seaver
A. D. Emmett	B. E. Livingston	William H. Welker
C. Stuart Gager	Mary G. McCormick	L. E. Wise
Geo. A. Geiger	G. M. Meyer	L. Lorande Woodruff

Sulfur in soils. That the sulfur in our soils, hitherto considered of little importance to the fertility of the same, is of vast importance, and is also being rapidly depleted due to improper methods of agriculture, is the gist of a bulletin published by the University of Wisconsin, embodying the results of experiments conducted by Mr. W. H. Peterson with Prof. E. B. Hart of the department of agricultural chemistry. Sulfur has been considered relatively unimportant as compared with the phosphorus and nitrogen contents of

soils. Tests made by Professor Hart and Mr. Peterson, however, show that low results were due to the analytical methods employed by the earlier investigators, and according to more accurate determinations the sulfur content of our soils is of vast importance. Continuous cultivation, in connection with insufficient fertilization, annually results in a heavy loss of sulfur. Combined with losses of sulfur through drainage and low original sulfur content of the soil, it appears that this loss can not be compensated by the sulfur obtained from the atmosphere. The surface eight inches of the normal soil yield only enough trioxide for about 100 normal crops of barley. The fact that the subsoil also has a low sulfur content, shows that the upward movement of capillary water can not bring much sulfur to the surface. In a word, it is necessary to apply fertilizers containing sulfur to maintain the crop yields of such soils. These experiments show that the sulfur content of a number of the common farm products, as previously determined, has been too low and that much sulfur trioxide is removed by crops from the soil—more than has been supposed. In fact, soils cropped continuously for half a century with infrequent applications of fertilizers, have lost as much as 40 per cent. of their original sulfur.

Book reviews. *Immune Sera.* A concise exposition of the main facts and theories of infection and immunity. By Charles F. Bolduan, M.D., bacteriologist, research laboratory, Department of Health, City of New York. Fourth edition. London: Chapman and Hall. New York: John Wiley & Sons. 1911. Pp. xi and 226. Price \$1.50.

In the present (its fourth) edition, Bolduan's book is slightly enlarged but fortunately still retains its small and handy size. The book makes no attempt at completeness but is intended as a general introduction to the complex subject of immunity and is one of the best books for the beginner in this field. From the enormous literature of the past few years the author has managed to summarize the most important advances. Some of the matter which he has picked out is open to criticism, but on the whole he has chosen very wisely. The most important of the new subjects are snake venoms and antivenins, anaphylaxis, the Wassermann and Noguchi reactions, the blood examination preparatory to transfusion, the meiostagmin

reaction, the Much-Holtzman cobra venom reaction in nervous diseases, the Weil venom test in syphilis and the antitrypsin determination. One small error may be pointed out. In discussing Weil's cobra venom test in syphilis, the author says "The reaction is possible in cases of jaundice whereas the Wassermann is not." The announcement a few years ago that jaundice interferes with the Wassermann reaction has not been confirmed, and in fact experience in thousands of jaundice cases has shown that jaundice only very rarely, if ever, interferes.

The discussion of all these new subjects is properly quite brief, because the chief value of the book is its lucid discussion of antitoxins, agglutinins, precipitins, bacteriolysins and hemolysins.

Education and Preventive Medicine. By Norman Edward Ditman, Ph.D., M.D. Octavo, 68 pp. Two appendices. Paper.

The continued demand for Dr. Ditman's paper, which originally appeared as a supplement to Volume X, No. 3, of the Columbia University Quarterly (June, 1908), has led to its second printing in separate form. The study takes up successively the economic loss from preventable diseases, as shown by data from ancient and modern records of epidemics and contagious sicknesses; the relief of suffering and the economic gain through preventive measures of established efficacy; the chief types of diseases still prevalent, but preventable, with some measure of their destructive effects; and the fundamental principles and institutions of preventive medicine. Specific methods are, of course, beyond the scope contemplated in the paper. It is Dr. Ditman's conclusion that not only has the application of beneficial methods of medicine been very slow, but often the application of scientific discoveries has been difficult to secure on account of popular ignorance and legislative bias and inertia. The extent of information now available for improving the conditions defined in his essay will be sufficient, if properly applied, to cause the effacement of a large proportion of the miseries of mankind. The purpose ably furthered by his paper is to increase not only the knowledge of the subject, but energy of application. In his belief the institution of a national board of health and of a school of preventive medicine, established in a great city, would be of great utility. (*Columbia Alumni News*, 1911, iii, p. 117.)

IV. COLUMBIA BIOCHEMICAL DEPARTMENT¹

Dr. E. D. Clark has been awarded a research scholarship at the New York Botanical Garden to assist him in investigations of certain phases of plant chemistry.

Professor Gies represents the Botanical Garden on the committee in charge of arrangements for the local reception and entertainment of the members in attendance at the Fifth International Congress on Hygiene and Demography to be held September, 1912.

Professor Gies, Drs. Eddy and Rosenbloom, Miss Seaman and Mr. Rose attended the recent meetings of the National Scientific Societies in Baltimore and Washington. Members of the staff made the following contributions to the programs of several of the societies.

William J. Gies. Modified collodion membranes for studies of diffusion.²

William H. Welker. Electrical apparatus for use with Benedict's method for the determination of urea.³

Charles H. Sanford and Jacob Rosenbloom. On the glycytryptophan and tryptophan tests for cancer of the stomach.³

E. D. Clark and F. J. Seaver. Studies on soils subjected to dry heat.²

E. D. Clark and R. A. Gortner. The chromogen and associated oxidases in the mushroom *Strobilomyces strobilaceus*.³

Emily C. Seaman. A course in sanitary chemistry: (a) Household; (b) Municipal and industrial.⁴

A. R. Rose. The influence of phytin on seedlings.²

A. R. Rose and J. T. Cusick. The influence of phosphorus compounds on the yield and composition of goat milk.²

A. R. Rose. The toxicity of phytin.²

Max Kahn. On the absorption and distribution of aluminium from aluminized food.²

Prof. Gies was a member of the committee which proposed amendments to the constitution of the American Society of Biological Chemists. He was appointed a member of a committee of

¹ Although it will be a policy of the BULLETIN to keep past workers in the Columbia biochemical laboratories intimately acquainted with local affairs, it is also planned to give similar attention, if possible, to all other biochemical laboratories. This plan, like many others connected with the BULLETIN, will be developed in the near future, after more immediate problems have been solved. See page 351.

² Joint session of the American Society of Biological Chemists with the Biological Section of the American Chemical Society.

³ Biological Section of the American Chemical Society.

⁴ American Home Economics Association.

five of that Society to consider the nomenclature, and report a classification, of the fats and fat-like substances. Dr. Gies's term as a member of the Council of the Biochemical Society recently expired. He was elected a member of its Nominating Committee.

The trustees of Teachers College, Columbia University, have created a school of practical arts, to comprise the present schools of household and industrial arts and the departments of fine arts, music and physical education. To this end there has been constituted a faculty of education, comprising the dean and the professors whose work is largely in education, who will direct the School of Education; also a faculty of practical arts, including the professors of fine arts, music, household arts, industrial arts, and hygiene and physical training. To this latter faculty is entrusted the development of the new School of Practical Arts, which will offer a new type of university education—a four-year course, comprising both academic and vocational courses. Professor Gies represents the department in the faculty of the new School of Practical Arts.

Dr. F. G. Goodridge has presented to the biochemical department a complete ultramicroscope outfit. The apparatus has been placed in position in the laboratory at the Medical School. It is a very valuable and serviceable addition to the departmental equipment and we are greatly indebted to Dr. Goodridge for his thoughtful generosity.

Miss Mabel C. Little has been appointed dietitian at the New York Polyclinic Hospital. Her appointment will not prevent her from completing the work on which she is now engaged in the department.

Miss Jessie A. Moore has recently been appointed an assistant in the chemical laboratory of the Rockefeller Institute for Medical Research.

At the last meeting of the Society for Experimental Biology and Medicine Dr. Herman O. Mosenthal discussed the results of his investigation of "Nitrogen and sodium chlorid excretion in experimental nephritis" which he has been conducting under the auspices of the Edward N. Gibbs Memorial prize fund.

Dr. Reuben Ottenberg was recently elected a member of the Society for Experimental Biology and Medicine.

Dr. Jacob Rosenbloom has lately accepted appointment to the position of assistant in bacteriology at the Mt. Sinai Hospital.

Prof. Wm. H. Welker has been elected a member of the Radium Institute of America, and also of the Harvey Medical Society.

Investigations of dental disease, which Professor Gies inaugurated two years ago with Dr. Lothrop's coöperation under the auspices of the New York Institute of Stomatology, are still in progress at the College of Physicians and Surgeons. The December issue of the *Journal of the (Allied) Dental Societies* contains the following papers on the subject (pp. 289-338): Attempts to improve the general method for the quantitative determination of sulfocyanate in saliva—*Elmer W. Baker and William J. Gies*. The oral microorganisms: A bacterio-chemical study of dental caries—*Alfred P. Lothrop*. On variations in the occurrence of nitrite in saliva—*Clayton S. Smith and Elmer W. Baker*. On the origin and significance of sulfocyanate in saliva—*William J. Gies*. The Committee on Scientific Research of the Dental Society of the State of New York recently invited Professor Gies to propose a plan of investigation of dental disease. He suggested the investigation of sulfocyanate in its possible relation to dental caries (*Dental Cosmos*, 1911, liii, p. 1324). The plan was approved and is now in process of execution by Professor Gies with the coöperation of Dr. Max Kahn in the biochemical laboratory at the College of Physicians and Surgeons.

Advanced students in the Department of Biological Chemistry have been appointed to fellowships and scholarships, as follows: Miss Louise McDanell, Research Scholar, Teachers College; Mr. Edward Gray Griffin, University Scholar in Organic Chemistry; Dr. Max Kahn, University Scholar in Organic Chemistry; Mr. Marston L. Hamlin, University Fellow in Organic Chemistry; Mr. Harold E. Woodward, Goldschmidt Fellow in Physical Chemistry.

Professor Gies continues to keep the laboratory at the College of Physicians and Surgeons open, and to guide investigators, at night by appointment. This plan has been followed for years in all cases where the interests of graduate students have prompted it.

EDITORIALS

The BIOCHEMICAL BULLETIN has been received with so many evidences of approval that we desire to make formal acknowledgment of our appreciation of the cordial and widespread interest which has been manifested in the new journal. Reception and future of the Biochemical Bulletin Members of the Biochemical Association, biochemical colleagues, librarians of universities and similar public institutions, and many others who cherish and promote the advancement of science, have tendered effective support. We have received far greater encouragement in our plans for the new journal than it would have been reasonable to expect. We accordingly rededicate our best efforts to the task of making the BIOCHEMICAL BULLETIN a "biochemical review" of special professional utility and of broadly scientific usefulness.

The influences which caused delay in the publication of the September number of the BULLETIN have hindered prompt appearance of this issue. Beginning with the March number, however, we shall be able quarterly to distribute copies during the last week of the regular month of issue. This number contains much of the material in our hands on December 31.

It will be impossible to publish in the BULLETIN all the manuscripts we have already received for the first volume. We shall adhere to our intention to issue annually but one volume of about 500 pages. Authors of papers for the June number and all succeeding issues are requested to endeavor to restrict their papers to 15 printed pages and to keep them within the bounds of 10 pages if it can be satisfactorily accomplished. We desire to present as much substance in as little space (and for as small an annual subscription) as possible. The coöperation of all contributors to these ends is earnestly invited. *Recrystallize your products several times,*

reject the mother liquors, and send us preparations of "tested purity"!

The constructive work involved in making the BULLETIN a "going concern" has left us little time or opportunity to mature our plans for strong "review" features in every issue. Steps to this end have, however, already been taken.

The reception accorded the BIOCHEMICAL BULLETIN has been so hearty and the manuscript for succeeding issues is so abundant, that promise of a successful career for this journal stimulates thoughts of the possible establishment of a *Biochemical Review* in charge of a body of editors from the biochemical profession at large. If such a journal could be established the BIOCHEMICAL BULLETIN would coöperate in the consummation of the project and might thereafter be devoted wholly to local affairs.

The BIOCHEMICAL BULLETIN is an ardent believer in biological chemistry as a science and an earnest advocate of it as a profession. We favor the continued independent existence of the American Society of Biological Chemists. Any movement intended to effect a merger of the American Society of Biological Chemists with any other organization to the detriment of biological chemistry as a profession would be opposed openly and candidly on these pages. We do not agree with the very recent dictum of an eminent American physiologist that "biological chemistry is an upstart" among the sciences, and that as such it should be adopted into a respectable family and brought up under a new name that would obliterate its past and give it a brilliant future. Five years ago when the American Society of Biological Chemists was organized, Professor Abel made a satisfying public statement of the reasons why biological chemists should perfect an independent professional organization. We commend Professor Abel's statement now to the attention of all who may be interested in seeking the dismemberment of the National Biochemical Society. (See the *Proceedings of the American Society of*

Biological Chemists, 1907, i, p. 2; *Science*, 1907, xxv, p. 140.) Dr. Alsberg lately called attention to some shortcomings of the American Society of Biological Chemists. We suggest that members of the Biochemical Society seriously consider what Dr. Alsberg has said. (See BIOCHEMICAL BULLETIN, 1911, i, p. 94.)

The medicinal value of arsenic in the inorganic form has long been established and its disadvantages and dangers are correspondingly well known. Within recent years various organic preparations of arsenic have been introduced with the medicinal value of arsenic compounds double purpose of extending the usefulness and restricting the dangers of arsenic. Ehrlich's theory of selective affinity has taken a prominent share in this development, resulting in the introduction of sodium arsanilate or "atoxyl," sodium acetyl-arsanilate or arsacetin, and lately of dioxydiaminoarsenobenzene, or "606," which has received the proprietary name "salvarsan." Still more recently the physicians' interest has gone back to a relatively well known organic arsenic compound, sodium cacodylate.

The history of atoxyl and arsacetin contains some very useful lessons. Their association with the names of Paul Ehrlich and Robert Koch caused them to be accepted as among the greatest discoveries of medicine, but in a relatively brief time it was found that their therapeutic value was restricted, and that they caused much more serious damage than ordinary arsenic. It is startling to reflect how many cases of total physical blindness, not to mention lesser consequences, have followed the moral blindness of those who applied the misleading name atoxyl—not toxic—to this preparation. As to salvarsan ("606"), it is too early to decide. Adverse reports continually appear. Further experience may bring to light many disadvantages which have been overlooked in the excitement and enthusiasm attending the introduction of a preparation which promises to be a great addition to materia medica.

We give on pages 270-324 abstracts of the symposium on Edema at the June meeting of the Biochemical Association and on pages

332-339 the Secretary's reports of the proceedings of the Association's second annual meeting in June and its first annual dinner in December. The scientific meetings of the Association have been profitable and pleasant for all concerned and promise to grow into important public functions. The first annual dinner was a revelation. *One hundred and twenty people present and all delighted!* The professionally cohesive force of such a happy event, the opportunities afforded for the renewal of old friendships and the formation of new ones, the stimulating effects upon the activities of the younger workers, and the democratic influences on the imagination and conduct of all concerned, cannot be overestimated. The following comment, in a note from one of the youngest members of the association, gives the substance of many similar significant expressions: "At this time I also wish to express my appreciation of the Biochemical Association dinner a short time ago. It is a great thing for us younger fellows to see and hear and speak with those who are well on in the race and who have 'made good.' Such a meeting is an inspiration in itself. I am looking forward already to the next one."

A crying need of botany in America today is deeper interest and greater activity in the *applications of chemistry* to its multifarious problems. *All* phases of botanical research are important, the chemical no less than the others. Where are the young botanists who fully appreciate the golden treasures that the biochemical mine contains? What is lacking in the present situation—training, or inclination, or understanding, or inspiration? Surely not facilities! What is the matter? The BIOCHEMICAL BULLETIN opens its pages to phytochemical papers and comment, and invites the interest and coöperation of botanists in its journalistic efforts as a missionary in this important field.

The discovery of the marked physiological actions of the suprarenal gland; the gradual isolation of the blood-pressure-raising prin-

ciple, *epinephrin*; the establishment of the constitutional formula of *epinephrin* and its final synthesis—these constitute one of the most attractive chapters in modern scientific exploration. The whole chapter, to which so many investigators have meritoriously contributed, is now fairly complete. The therapeutic availability of this important substance is now also fairly well defined, as also its limitation by the poor absorption and prompt destruction of the alkaloid when it is administered through the ordinary channels.

The reading matter furnished by advertising houses did not follow these discoveries. Early in the current year the circulars wrapped about some of the most important commercial brands still stated that *epinephrin* could be successfully administered by mouth. Another circular claimed that the recurrence of cancer could be prevented by the external, or even by the internal, use of *epinephrin* preparations. It is unsafe to rely on the advertising matter of even the better manufacturers, even when it relates to substances of established intrinsic merit.

The suprarenal industry is also unsatisfactory from another standpoint, namely: the variability of product. This may not be of very great practical importance in the external use; but when given intravenously, it is most important, since *epinephrin* may cause alarming effects when introduced in this way. This variability is largely unintentional. It is due partly to the fact that the various manufacturers employ different methods of standardization; and partly to the deterioration of the products with age. Both of these difficulties can be overcome, and there are indications that most of the prominent manufacturers will coöperate for this purpose.

We refer the reader to an editorial in the BIOCHEMICAL BULLETIN (1911, i, p. 154) on the need of non-proprietary, descriptive names for medicinal substances. It would be well, it seems to us, to emphasize to students that all the various commercial suprarenal preparations owe their activity to a definite chemical substance—*epinephrin*.

The Eighth International Congress of Applied Chemistry (Washington and New York, September, 1912) was invited to

this country by the President of the United States at the solicitation of more than 4,000 American chemists. The very least that biological chemists can do is to join the Congress. The membership fee of \$5 is wholly nominal when compared with the benefits of such membership. The twenty-four sections of the Congress will together present upwards of 1,000 papers, of which more than 300 were promised by American chemists by December 31, 1911; the printed report of the Congress is expected to contain upwards of 5,000 pages, and will be sent free of charge to all members; to non-members the price will be \$20. Members who are unable to attend the meetings will nevertheless have for reference and use the final records thereof.

Further information and application blanks for membership can be had from Bernhard C. Hesse, Secretary of the Congress, 25 Broad Street, New York. The members of the Executive Committee of the Section of Physiological Chemistry and Pharmacology are John J. Abel, *President*; William J. Gies, *Vice-President*; John A. Mandel, *Secretary*; Reid Hunt and Thomas B. Osborne.

The public is generally ignorant of the difference between *antiseptics* and *disinfectants* (*germicides*), and even many members of the medical profession confuse these terms. Manufacturers of spurious disinfectants have taken advantage of this confusion, wittingly or unwittingly; thereby menacing the safety, not only of the individual, but of entire communities.

An *antiseptic* is an agent that, used under suitable conditions, prevents the multiplication of bacteria without necessarily destroying them; a *disinfectant* (*germicide*) is an agent that, employed under suitable conditions, completely and quickly *destroys* bacterial life.

Disinfectant agents may in very dilute solutions act as antiseptics; and antiseptic agents, *if given a sufficiently long time to act*, might ultimately succeed in killing off bacteria, either by a process of starvation or by direct chemical means.

These agents have their own special spheres of usefulness. Antiseptics are used for the preservation of organic liquids, such as serums, urine, etc. In surgical procedure boric acid, phenol (1 : 100), mercury chlorid (1 : 20,000), as used in the treatment of wounds and suppurating processes, exert most largely an antiseptic action; they do not destroy the bacteria at the point of the attack, but check the proliferation of the germs in the secretions or exudates and prevent bacteria from the outside establishing themselves in the wound, and quite likely act upon the tissue cells, stimulating them to resist and overwhelm the invading microorganisms.

While disinfectants are powerful destroyers of bacterial life they act injuriously on living tissues, so that their application to the body is limited most largely to the cauterizing of infected wounds, or to the destruction of small superficial infected areas. Their widest field of usefulness lies in the disinfection of bacteria-laden excretions or secretions; *e. g.*, the employment of milk of lime, "chlorid of lime," formaldehyd and carbolic acid in the disinfection of the stools of typhoid and cholera patients, the urine in the typhoid and Malta fevers, tuberculous sputum and the like. In all these cases an insufficient strength of the destructive agent is little better than none at all. The hands of the surgeon can be superficially disinfected by the application of strong solutions of mercuric chlorid, iodine, potassium permanganate and oxalic acid, etc., whereas under these conditions the use of *antiseptics* would be entirely without avail and dangerous, in that their use might lead to the neglect of really effective measures. Rooms infected by the germs of diphtheria, scarlet fever or tuberculosis can be rendered germ-free by the employment of such powerful destructive gases as formaldehyd and sulfur dioxid; not by the fumes of some delightfully or noxiously smelling well-advertised preparation, or by the sprinkling of so-called "chlorids" or other trash about the room.

It cannot be too strongly emphasized that the employment of either an antiseptic or a disinfectant (germicide) is a matter of the greatest importance and that physicians should be prepared to give very definite instructions in regard to this matter to the patient or the nurse.

The student should be taught the essentials of the method for determining antiseptic and disinfectant strength and it should be impressed on him that the claims for a proprietary article should never be seriously considered without verification by a competent and unbiased investigator—one who knows the pitfalls to be avoided in the making of the test, wherein one less skilled may fall. Many a proprietary article on the market will be found to possess relatively little power as a disinfectant, but may have the qualities of an excellent antiseptic. Such a preparation may be very useful in its proper field; but reliance on it as a germicide would be disastrous.

The award of the Nobel prize in chemistry to Mme. Curie is an honor as signal as any ever conferred. It is a tribute to Mme. Curie's scientific attainments that is shared generally by her sex and will advance woman's cause everywhere.

Mme. Curie Prof. and Mme. Curie shared in the prize for physics in 1903 with Becquerel, the discoverer of the Becquerel rays. It is most notable that anyone should win such fame in two branches of science as to be awarded an important prize in each.

If a little knowledge is dangerous, where is the man who has so much as to be out of danger?—*Huxley*.

The successful worker must have the spirit of play in his heart, and the successful man is only a boy with a man's experience.—*Hughes*.

Seedlings What do the clinicians of to-day—even the most learned—know of respiration? Next to nothing! And this little is mostly wrong.—*Henderson*.

I have learned three things in Paris: Not to take authority when I can have facts, not to guess when I can know, and not to think that a man must take physic because he is sick.—*Holmes*.

I know nothing that is so conducive to a cheerful optimism in these present days as the pursuit of science. The laboratory is the habitation of buoyancy, enthusiasm and hope. Its occupant has no moral right to be despondent, and, if he is so, there is surely something pathological in the activities of his brain-cells. Actually, however, one rarely meets with a pessimistic man of science.—*Lee*.

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(Abbreviations: C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51. **ELEMENTARY ORGANIC CHEMISTRY.** Introductory to courses 101, 102 and 104. (*Required of first year students of medicine.*) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, and Mr. Smith.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101-102. **GENERAL PHYSIOLOGICAL CHEMISTRY.** *A course in the elements of normal nutrition.* (Teachers College, School of Household Arts.) L, 1 hr. R, 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Miss Seaman and Mr. Miller. (This course is designated "H. A. 25" in the Teachers College Announcement.)

This course is designated "S—H. A. 25" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies and Miss Seaman.

104. **GENERAL PHYSIOLOGICAL CHEMISTRY.** *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R, 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, Dr. Clark, and Messrs. Smith and Rose.

This course is designated "S—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Mr. Smith.

201-202. **CHEMISTRY OF NUTRITION.** (School of Pharmacy. *Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

203-204. **GENERAL BIOLOGICAL CHEMISTRY.** *Specially adapted to the needs of secondary school teachers of biology.* L, 1 hr. Lw, 4 hr. Dr. Eddy.

205-206. **ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION.** (Teachers College, School of Household Arts.) L, 1 hr. Lw, 5 hr. Prof. Gies and Miss Seaman. (This course is designated "H. A. 125" in the Teachers College Announcement.)

Courses in Nutrition (continued)

207-208. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS. L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Lothrop.

209-210. NUTRITION IN HEALTH AND DISEASE. L, 2 hr. Prof. Gies.

211-212. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies, Mr. Rose, and Dr. Clark.

213-214. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies.

215-216. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Welker, Drs. Lothrop and Clark and Mr. Rose.

TOXICOLOGY

217-218. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. Lw, 6 hr. Prof. Gies.

BOTANY

219-220. CHEMICAL PHYSIOLOGY OF PLANTS. (New York Botanical Garden.) L, 1 hr. Lw, 5 hr. Prof. Gies and Dr. Clark.

BACTERIOLOGY

221-222. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Clark.

SANITATION

105. SANITARY CHEMISTRY. (Teachers College, School of Household Arts). L, 1 hr. Lw, 3 hr. Professor Gies, Miss Seaman and Dr. Clark. (This course is designated "H. A. 26, a" in the Teachers College Announcement.)

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301-302. BIOCHEMICAL SEMINAR. 1 hr. Prof. Gies.

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Summer session courses are mentioned in the foregoing references to Courses 101-102 and 104. Prof. Gies will have charge of both courses next summer. He will also conduct a special lecture course in nutrition. The laboratories will be open, during the summer session, to advanced workers for research. (See the BIOCHEMICAL BULLETIN, 1911, i, p. 150.)

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Synthese der Zellbausteine in Pflanze und Tier. Lösung des Problems der künstlichen Darstellung der Nahrungsstoffe. By Emil Abderhalden. Pp. 128. Unbound: 3.60 Marks; bound 4.40 Marks. Julius Springer, Berlin.

Schutzfermente des tierischen Organismus. Ein Beitrag zur Kenntnis der Abwehrmassregeln des tierischen Organismus gegen körperl., blut- und zellfremde Stoffe. By Emil Abderhalden. Pp. 110. Unbound: 3.20 Marks; bound: 3.80 Marks. Julius Springer, Berlin.

Physiologisches Praktikum. Chemische und physikalische Methoden (with 271 figures in the text). By Emil Abderhalden. Pp. 283. Unbound: 10 Marks; bound: 10.80 Marks. Julius Springer, Berlin.

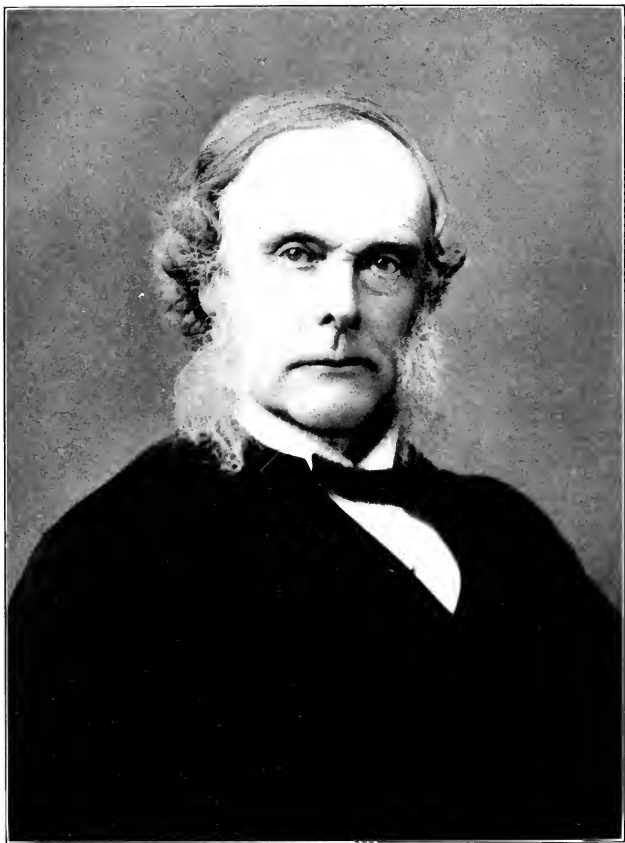
Principles of human nutrition. A study in practical dietetics. By Whitman H. Jordan. Pp. 450; \$1.75. Macmillan Company, New York City. *See review:* This issue of the BIOCHEMICAL BULLETIN, page 510.

Methods of organic analysis. Second edition, rewritten and enlarged. By Henry C. Sherman. Pp. 407; \$2.40, net. Macmillan Company, New York City.

Zeitschrift für Gärungsphysiologie. (See page 505.)

Zentralblatt für die gesamte innere Medizin und ihre Grenzgebiete. (Page 504.)

This issue of the BULLETIN is, in effect, the March and June numbers combined (pages 371-528). The June number will be issued late in July. It will consist chiefly of personalia, with title page, table of contents and an elaborate index, in completion of Volume I.



Joseph Lister

BIOCHEMICAL BULLETIN

VOLUME I

MARCH, 1912

No. 3



IN MEMORIAM

JOSEPH LISTER

Born April 5, 1827. Died February 11, 1912

Although a believer, with chemists generally of his day, that putrefaction and wound infection were caused by the presence of atmospheric oxygen, Lister was the first to realize the great surgical significance of Pasteur's work on fermentation.


By his acceptance of Pasteur's discovery of the bacterial origin of putrefaction, and by his practical application of it to the principles of wound treatment, Lister, with his "acide phenique," founded antiseptic surgery.

His modest but unflinching insistence upon the validity of his new methods finally established them beyond a doubt and thus prepared the way for the invention of the aseptic surgical technique of today.

Aided by anesthesia and based on disinfection—biochemical agencies of profound surgical import—Lister's methods became the most powerful factor in the development of modern surgery.

By his revolution in technical procedure Lister advanced every branch of the healing art and brought relief of suffering with prolongation of life to all parts of the world.

He lived esteemed and died revered the world over, an unforgettable benefactor of mankind.



JUL 27 1912

WALDEMAR KOCH

Herman Koch, a mining engineer of international reputation, lived in Clausthal, Hannover, Germany. His father, his grandfather and his grandfather's father had been mining engineers before him. One of his nine sons was Robert Koch, the great bacteriologist; another was Hugo Koch, also a mining engineer; another, Arnold Koch, came to this country in 1867 with letters of introduction from Alfred Nobel, who was a friend of Herman Koch. Arnold Koch settled in St. Louis, where his only son, Waldemar, was born April 8, 1875.

The first part of Dr. Koch's college life was spent in Washington University, St. Louis, but his last year he spent in Harvard, from which he received his undergraduate degree, and two years later, in 1900, the degree of Ph.D. in organic chemistry. He was then for one year assistant in physiology in the Harvard Medical School with Professor Porter. He began at that time the study of the chemistry of the nervous system, which he continued until his death. He came to the University of Chicago as an associate in physiological chemistry in 1901, and with a short interregnum spent in teaching pharmacology and physiological chemistry in the University of Missouri, he remained in the University of Chicago continuously thereafter, where at the time of his death he was associate professor of pharmacology. He was for a time with Schmiedeberg in Strassburg; and during his vacations he worked for several years, part of the time under a grant from the Rockefeller Institute, in the laboratory of Dr. Mott in the Claybury Asylum for the Insane, near London; afterwards for one season he was on the staff of the new hospital for the insane at Long Grove, near London; and for the past year he had been connected, also, with the Wistar Institute of Anatomy in Philadelphia. In these various institutions he had unusual opportunities, which he utilized to the utmost, for the study of pathological and normal nervous material.

Dr. Koch's work on the chemistry of the nervous system is



Waldemar Korch.

known to all physiological chemists. The chemistry of the brain is a very difficult field and the separation of the lipoid substances is still hardly possible. He spent the greater part of these years in devising accurate methods of quantitative analysis. These methods had been so perfected that he had secured more complete and more accurate quantitative analyses of nervous tissue than any hitherto made. By means of these methods he was attacking the problem of the differentiation of the brain during growth; the distribution of various substances in different parts of the brain; variations in composition during disease; and the differences between the brains of different animals. He was also engaged in separating carefully the various lipoids, such as kephalin and lecithin, and in examining their composition. He showed the very important fact that kephalin exists as a potassium salt, whereas lecithin has more of an affinity for sodium. His method of purification of the lipoids by precipitation with chloroform and hydrochloric acid was extremely useful. Among his other important contributions must be mentioned his work on the behavior of lecithin and kephalin emulsions towards various salts, anesthetics and drugs, work which showed one way in which these substances might influence irritability. He discovered, also, that the brains of persons having the very obscure insanity, dementia præcox, contained less of a certain sulfur fraction than usual, and in his further examination of the sulfur distribution in the brain he isolated a lipoid-sulfur compound of very interesting nature. He had prepared a considerable quantity of this compound and he was engaged in studying its nature at the time of his death.

Dr. Koch's interest from the first had been in the problem of the action of drugs on the nervous system. He taught pharmacology almost from the time of his graduation. He fitted himself for his duties as a teacher by taking the regular medical courses in pathology, anatomy, embryology and many of the clinical courses, as well as by studying with Schmiedeberg in Germany. He thus had a very unusually broad training and was able to look at his subject from all sides, the chemical, the biological, and the clinical. There are very few men in pharmacology to-day, who possess his extensive knowledge and his sane, scientific and broad point of view.

The work he was doing on the nervous system he regarded as fundamental work in pharmacology, necessary before any real science of pharmacology could be constructed. He had just reached a point when the direct application of his methods to the solution of the problem of how drugs combine with nerve cells could be begun. To die at such a time was particularly cruel. Had he lived a few years longer his recognition as one of the leading pharmacologists of the world would undoubtedly have been assured. His work, like that of his uncle, Robert Koch, was very thorough and exact, and he proceeded in logical order to overcome one difficulty after another.

Dr. Koch's personality won him many friends. He was a true, loyal and courageous friend, entirely honest and of sane judgment; he avoided making enemies, as far as possible. He was fond of out of doors, and loved the hills, rivers and fields, and tramping in the dunes near Chicago was his main recreation. He had a keen appreciation of what was fine in music and in art. His was an open, frank, kindly nature, considerate of others and slow to anger.

His death by pneumonia, February 1st, at the early age of thirty-six, was an irreparable loss to his friends, to his University and to science.

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ALBERT P. MATHEWS.

University of Chicago.

THE DEVELOPMENT OF BIOCHEMICAL INSTRUCTION AND RESEARCH IN NEW YORK CITY¹

JOHN A. MANDEL

(*New York University and Bellevue Hospital Medical College*)

On carefully studying the condition of biochemical instruction and research in New York City, at the present time we find that we have the four medical schools with well-equipped laboratories for instruction of students and for research work; the Rockefeller Institute for Medical Research, which is the most prominent institute of its kind in the world; the several large hospitals, such as Bellevue, New York, Roosevelt, Presbyterian, St. Luke's, Mt. Sinai, St. Vincent's and many more, all of which have more or less extensive laboratories for chemical and biological investigations. Incidentally, I should say that the recently erected laboratory building in connection with the Bellevue and Allied Hospitals, and now in progress of organization, is the largest and most complete plant of its kind in the world. Besides these we have a number of laboratories in connection with the Board of Health, the Sage Foundation, Montefiore Home, and other institutions, as well as a number of laboratories maintained by private individuals, the most prominent being the private laboratory started by the late Dr. C. A. Herter in 1892.

The directors of all these laboratories are experienced men in the field of biochemical research, and these facts and others make New York City without question the real center of biochemical research in this country. These conditions are truly very gratifying and instructive, but the most remarkable fact is that *all this development has taken place within the last fifteen years*. Before 1896 there existed no laboratory in New York City where physiological chemistry was solely taught and where biochemical research was carried on in the present-day sense.

¹Read at the first annual dinner of the Columbia University Biochemical Association, December 13, 1911 (see page 334).

The College of Physicians and Surgeons, which was chartered in 1807 and adopted as the medical department of Columbia College in 1860, has on its rolls the names of Mitchell, De Witt, Macneven, Dana, Torrey, St. John and Chandler as professors of chemistry, and Smith, Post, Watts, Alonzo Clark, Dalton and Curtis as professors of physiology. The instruction in these departments was given entirely by didactic lectures and no laboratory work was done by the students until Chandler took charge of the instruction in 1872. Incidentally, I might mention the fact that a student (Colton) under Dr. Torrey at the old Crosby Street School, after witnessing the anesthetic effects of nitrous oxide during the lecture, proposed to make some money for his medical education by giving some public exhibitions of the effects of this gas. While demonstrating this at Hartford, Conn., one of the audience, a dentist, Dr. Wells, was impressed by the exhibition and asked Colton to administer the gas to a patient while he removed a tooth. This was the first application of laughing gas to dentistry and surgery.

The Medical Department of New York University was organized in 1841 and the chair of chemistry has been occupied by John W. Draper, John C. Draper and Rudolph Witthaus and the chair of physiology by John W. Draper, Henry Draper, John Arnold, Lewis A. Stimson, and William Gilman Thompson. Of these men John W. Draper had a strong influence upon medical education and scientific research and he made important contributions to both chemistry and physics. He was a prolific writer and among his publications I should mention a Text Book of Chemistry, a Text Book of Natural Philosophy, a treatise on the forms that produce the organization of plants, a treatise on physiology, a History of the Intellectual Development of Europe, Thoughts on the Civil Policy of America, History of the Rebellion, Conflict of Religion and Science. His work on glandular action, endosmosis and radiant heat are classical and his application of the daguerreotype process to taking portraits is immortal. No laboratory instruction was given in this institution until about 1868 and no special instruction in physiological chemistry was given until Rudolph Witthaus was appointed in 1876.

The Faculty of the Bellevue Hospital Medical College was

elected in 1861 and in 1898 the majority of its faculty joined with some of the members of the University Medical School, forming the University and Bellevue Hospital Medical College. In the old Bellevue School we find Austin Flint professor of physiology and R. Ogden Doremus professor of chemistry. It was at this school that laboratory instruction in chemistry was first given to students. This was in 1863 and after this date laboratory work became a part of the curriculum in the other schools.

It is surprising that it should have taken such a long time for the appreciation, in this country, of the value of practical laboratory work in the instruction of medical students. We know that the great genius, Liebig, opened his laboratory at Giessen for students in 1824, with two applicants, and that in 1833 and 1839 he was obliged to enlarge his laboratory facilities on account of the large number of native and foreign applicants. At this time most of the German universities followed Liebig and built laboratories, and had more or less success in attracting students; but it took New York thirty to forty years to be impressed with the fact that laboratory teaching was of the very greatest importance.

In 1881, when I first came to Bellevue, the chemical instruction consisted of sixty lectures and twenty recitations a year. Most of these lectures were on chemical physics, being extensively illustrated, while fewer lectures were given on pure inorganic and organic chemistry. A disproportional number of lectures was given on toxicology and medical jurisprudence. The laboratory work consisted of twenty hours for each student devoted to bedside testing for metallic and alkaloidal poisons, and the clinical examination of urine, qualitative and quantitative. A very similar condition existed in the other medical schools.

In looking over the past records of the literature, I cannot find any contribution of importance to the biochemical sciences by any of the incumbents of the chairs of chemistry in any of the medical colleges up to 1896. Their time was devoted to teaching in one or more institutions or they were interested in toxicological examinations of various kinds or other legal cases involving chemical knowledge and expert testimony. I do not mean to infer that they did not have a pronounced influence upon the municipal welfare and the

hygienic conditions of the communities in which they lived, but they contributed little towards the advancement of the science that interests us so much, namely physiological chemistry. At the time when Chandler, Draper and Doremus occupied important chairs of chemistry in New York City, there were men like Hoppe-Seyler, Baumann, Salkowski, Voit, Pettenkoffer, Drechsel, Hofmeister, Kühne, Pflüger, Wurtz, and many others working abroad, who directly or indirectly were influenced by the great Liebig, and his great biochemical researches, and the laboratories organized by these men are at the present time the chief fountains of biochemical knowledge.

At this time I wish to pay tribute to the scientific work of one who was professor of physiology at the Bellevue Hospital Medical College until 1898 and then accepted the chair of physiology at the Cornell Medical School, which position he occupied until 1908, namely, Dr. Austin Flint. Since 1855 he has published sixty-three papers and essays on various medical and biochemical topics, among which I wish to call your attention to a paper entitled "The influence of the excessive and prolonged muscular exercise on the elimination of effete matters by the kidneys," published in the New York Medical Journal in 1870 and republished in an extended form in the Journal of Anatomy and Physiology in 1877. These observations were made on the pedestrian Weston and form the first investigation on the influence of exercise upon the excretion of nitrogen by comparing the nitrogen eliminated with the nitrogen of the food. Another paper of interest, published in 1862, is one on *stercorin*, a derivative of cholesterol, formed in the intestine and rediscovered by Bondzynski and Humnicke in 1896 and called *koprosterin* by them. Among others of interest I find one, "On the organic nitrogenous principles of the body, with a new method for their estimation in the blood," published in 1863; experiments undertaken for the purpose of reconciling some of the discordant observations on the glycogenic function of the liver in 1869; various papers on the nerves and their action; treatment of diabetes, etc., etc. Not only was he an enthusiastic experimenter but a very forceful teacher.

With this short review of the past, we must, I am sure, feel happy that we are living in the present, when the conditions and

facilities for biochemical research are developing and our efforts appreciated by the medical and lay communities. I do not think of anyone who has contributed more to this condition of development than the guest of this evening, Professor R. H. Chittenden. By the organization of the physiological chemical laboratory at the Sheffield Scientific School about 1875, the first in this country, and by the systematic and extensive training given to a large number of students, he has emphasized the importance of this branch of chemical research and has made possible the development by supplying well-trained men to act as organizers in other institutions. For this reason we all owe Professor Chittenden a deep debt of gratitude and we believe that his influence will be felt for many years to come.

THE STUDY OF ENVIRONMENT¹

WILDER D. BANCROFT

(*Cornell University, Ithaca, N. Y.*)

It may be asked why a chemist should speak on such a subject as this. One reason is that it is really a chemical problem, and the biological chemist can, or should, handle it better than the straight biologist. Another reason is that the biologists and naturalists know too many facts and are swamped by them. A hypothesis has no chance of developing into a theory because it will be crushed at once by an overwhelming mass of apparent contradictions. Consequently the biologist is forced, in self-defense, to go on collecting more facts and burying himself deeper. The chemist does not know as many facts as he should; but he knows the Theorem of Le Chatelier, that a system tends to change so as to minimize an external disturbance.² Knowing this is a universal law, he has a definite basis from which to start. Of course the chemist may misapply the law and he may, in his ignorance, overlook disturbing factors which ought to be taken into account; but a poor working hypothesis is better than none, and a good one is invaluable.

The first question that the chemist asks himself is whether the study of the effect of environment has been carried on in a satisfactory way, and my object is to call your attention to the way in which I think that one should study the effect of environment, and also to the reasons why I think so.

When studying the effect of environment, we should distinguish sharply between: the direct effect of external conditions involving no adaptation; the adaptation of an organism, during its lifetime, to new external conditions; and the possible inheritance of adaptations. In most cases the biologist has not kept these things separate. A few illustrations will make clear what I mean. We will begin with the effect of external conditions where there is no adaptation. A blow with an axe may cut off a branch. In this case we get the direct result and no adaptation. If we deprive a man of

¹ Abstract of an address delivered at a meeting of the Columbia University Biochemical Association, in Rumford Hall of the New York Chemists' Club, March 15, 1912.

² Bancroft: *Jour. Am. Chem. Soc.*, **33**, 91 (1911).

food and drink for a sufficient length of time, he will die. That is the direct result of the new conditions and does not involve any adaptation. If we give a suitable man a suitable amount of a suitable alcoholic drink, he will become drunk. A more interesting case is that of the action of sunlight. The direct action of light is to tend to bleach out any color which absorbs the light and we find that an over-intense light does tend to bleach plants,³ even though these plants would have been more bleached if they had grown in the dark. These illustrations are so obvious that they probably seem superfluous; but they are really obvious only when put in this form. For instance, in a presidential address before the American Society of Naturalists, MacDougal⁴ discusses the question of adaptive response to the environment and says: "Further, a critical examination fails to disclose any theoretical considerations or any actual facts which would connect inevitably the somatic response with the nature of the excitation, outside of the specialized tropisms in which specific reactions are displayed. Even in these the adjustment is of such nature that a mechanism especially responsive to contact, tendrils, for example, responds in the same manner to temperature variations, to which the movements are in no sense accommodations or adjustments." MacDougal evidently thinks that one ought to show that the change in the clinging power of the tendrils with changing temperature is directly beneficial. Of course, this really comes under the head of the direct action of external conditions. It is a necessary consequence of the nature and structure of the tendrils. One might just as well insist that one prove that the shortening of a fishing line, when wetted, is an adaptation acquired because it is beneficial to somebody or something. Mistakes like this retard progress.

Under the heading of adaptation of the organism during its lifetime to new conditions, we can take up this same disturbing factor right over again. I do not know of any plant which develops armor-plate as its way of meeting blows with an axe. It is often said that the development of thorns has come about as a protection against animals; but I don't imagine that anybody really believes that nowadays. There is one apparently beautiful case of adapta-

³ Boehm: Jour. Chem. Soc., 34, 238 (1878).

⁴ MacDougal: Science, 33, 95 (1911).

tion which also will not stand investigation. When dandelions first bloom in the spring on a golf course, they come up eight to ten inches high and are promptly cut down by the lawn-mower. The succeeding crops have very short stems and are unfortunately not damaged by the mower. On examination, the length of the stems proves to be determined much more by the intensity of the light than by the danger from the lawn-mower. The real way in which a tree adapts itself to onslaughts with an axe, is by developing buds back of the point of attack.

If we subject a plant to starvation and thirst by planting it in a poor soil in a dry country, the adaptation consists in a larger root development and in modifications which cut down the evaporation. If we continue to give our hypothetical man alcoholic drinks, he will probably adapt himself to the new environment to the extent that it will take more liquor to make him drunk. The response to the bleaching action of the light is the increased production of chlorophyll. In all cases, it is important to make certain what is cause and what is effect. An interesting instance occurs with *Proserpinaca palustris* as studied by Burns.⁵ This plant is easy of culture both on land and in water. The "land type" of leaf is lanceolate with serrated margins while the "water type" is finely divided, with a central rib and from three to five filamentous divisions on each side. A careful study brought out the fact that "the water environment is not the cause of the division of the leaf. Nor does it depend upon light, temperature, gaseous content of the water, or the contact-stimulus as such. The only conclusion that seems justified by my experiments seems to be that *Proserpinaca palustris* has two forms—an adult form and a juvenile form. Under good vegetative conditions it has a tendency to produce the adult form with the entire leaf, blossom and fruit; under poor vegetative conditions it has a tendency to produce the juvenile form with the divided leaf. And furthermore, a reversion to the primitive form may be caused by unfavorably influencing the vegetative conditions."

It must also be remembered that any direct development of any organ in response to external stimulus is going to be accompanied by a more or less general rearrangement throughout the system. These other changes are secondary ones and may or may not be

⁵ Burns: *Annals of Botany*, 18, 579 (1904).

beneficial to the organism as a whole. Developing the muscles in itself is a good thing; but if it puts a strain on the heart, the sum total of the effects may be bad. A striking instance of this occurs in the case of alcohol. The man, who can carry his liquor better than formerly, has responded beneficially as far as that particular aspect of the external stimulus is concerned; but it would be foolish to claim that hard drinking ought, therefore, to be beneficial in all ways. And yet this is practically what MacDougal⁶ does. "It is unanimously agreed that organisms, plants as well as animals, change individually in aspect, in form and structure of the organs, in functionation and habit as they encounter swamps, saline areas, gravelly uplands or slopes, climatic differences identifiable with latitude or elevation, and other physical and biological factors. It is assumed that these somatic alterations are accommodative and adaptive, making the organisms more suitable for the conditions which produce the changes. Such an assumption is an over-reaching one. Any analysis of the changes which an organism undergoes after transportation to a new habitat will disclose one or a few alterations which might be of advantage in dealing with the newly encountered conditions, but with these are many others, direct, necessitous, atrophic or hypertrophic as to organs which have no relations whatever to usefulness or fitness."

It is quite clear from this quotation that MacDougal has never had a clear conception of the problem as it presents itself to the chemist. It is also clear that no satisfactory progress can be expected so long as people consider total changes only, and do not differentiate them at least into primary and secondary changes. It is very much to be desired that some ambitious young man should go over the literature in regard to the effect of environment on the organism during its lifetime, discussing it critically and from a rational point of view. It would not be so amusing as trying to produce mutants artificially; but it is quite possible that the net result to science might be greater.

The proper time to study the problem of the inheritance of adaptations is after we have cleared up the question of adaptations during the lifetime of the organism. The fates have forced our hand and consequently it is necessary to discuss some portions of

⁶ MacDougal: *Science*, 33, 95 (1911).

the problem of heredity from the viewpoint of the chemist. The view of the biologist is, or ought to be, that each generation always varies spontaneously from the preceding one to a greater or lesser extent, and that these variations are reproduced more or less completely in the succeeding generation. By the survival of the fittest we eventually get a race which is better adapted to the local conditions than the one with which we started. The view of the believer in the Theorem of Le Chatelier is that external conditions tend to produce such changes in the organism that the next generation tends to vary in such a way as to be more adapted to local conditions. By the survival of the fittest and by the continued actions of the external conditions, we eventually get a race which is better adapted to local conditions than the one from which we started.

It might as well be admitted frankly that the facts as they stand are overwhelmingly in favor of the point of view which I have ascribed to the biologists. This may be due to an error in applying the Theorem of Le Chatelier. All that our theorem tells us is that such and such a change tends to take place; but the means may be insufficient to accomplish the end. If I butt my head against a stone wall, I tend to knock the wall over; but the wall is not likely to fall under these circumstances. We have at least one instance where things seem not to go as they should in the domain of inorganic chemistry. A strong magnetic field should have a distinct influence on the rate of reaction of iron salts and yet it apparently does not.⁷ We do not know whether the magnetic field was not strong enough or whether some overlooked factor has nullified the effect that we sought. It is possible that a tendency to inherit character does exist; but that the actual result is negligible. It is always possible to save one's face in this way; but it does not appeal to me. I believe in carrying the war into Africa, so I raise the question as to how satisfactory the biologist's experiments really are.

A few years ago one would have said that it was quite impossible to make the scarlet tanager and the bobolink keep their breeding plumage throughout the year and still more impossible to make them moult from breeding plumage direct to breeding plumage, and yet both of these things have apparently been done by Beebe.⁸ This

⁷ M. Loeb: *Am. Chem. Jour.*, **13**, 9 (1891).

⁸ Beebe: *The American Naturalist*, **42**, 34 (1908).

is a good instance of how unsafe it is to predict from negative results. There is a much more serious charge to be brought against the biologist. He rules out all cases in which the acquired character is not permanent for a number of generations.⁹ In this way he bars out practically all the data which could be used against him. In general we can say that inheritance is a hysteresis phenomenon and that changes which take place readily disappear readily. It is safer not to apply this to mutants for the present, though they can hardly be classed as changes which take place readily because there is grave doubt whether anybody has ever produced a mutant at will. They have seen mutants appear but that is a very different thing.

If a plant adapts itself readily to new conditions, it will presumably revert quickly when brought back to the old conditions and consequently people say that there has been no inheritance of acquired characters, whereas the whole thing may be merely a matter of definition. On the other hand an organism which does not react readily to the new conditions will require experiments extending over many generations in order to show results. With organisms of this class, there is practically no inherited adaptation within the time usually covered by the experiments. We get admirable illustrations of this in the work of Naegeli and of Zederbauer.¹⁰ Naegeli took plants up the mountains and found that they assumed alpine characteristics which disappeared when seeds of the plants were brought back again to Munich, wherefore he said that the acquired characters were not inherited. There is a beautiful logical dilemma here. Unless one knows where the plants originally came from, one cannot tell whether it is the Munich characteristics or the alpine characteristics which are inherited while the others are not. In Zederbauer's experiments, "when seeds were taken from plants on the elevated plateau where their ancestors may have been for many years or many centuries (perhaps as long as 2,000 years) and sowed at Vienna and at other places, it was found that in four generations the leaves lost their xerophytic forms and structure, but the other characters were retained within the limits of variability. The stems showed an increase in average length of 1-2 cm., the roots changed as much, but the reproductive branches and floral

⁹ Cf. MacDougal: *Science*, 33, 96 (1911).

¹⁰ Cf. MacDougal: *Science*, 33, 98 (1911).

organs retained their alpine characters. The slight modifications undergone by these features were seen to reach a maximum and to decrease in the latest generations cultivated. The structural changes and implied functional changes are originally direct somatic responses; there is no escape from the conclusions that the impress of the alpine climate on the soma has been communicated to the germ-plasm in such a manner as to be transmissible, and the suggestion lies near that repeated and continued excitation by climatic factors may have been the essential factor in such fixation." It is rather curious to see MacDougal treating this as a case of direct effect of environment when it is quite as possible to explain it on the assumption that the alpine characters had become fixed in the alleged two thousand years by spontaneous, indiscriminate variation and the survival of the fittest.

If we drop the arbitrary definition that acquired characters can be considered as inherited only if they come true for half a dozen generations after the organism has been restored to the original conditions, the facts will be tremendously against the biologist's point of view. If we drop this arbitrary definition, as I think we should, we are confronted by the difficulty of distinguishing, in certain cases, between inheritance and adaptation during life. Le Clerc and Leavitt¹¹ consider that climatic conditions are of tremendous importance in regard to wheat.

"Too much has been taken for granted regarding the influence of heredity in plants. Without detracting from the power which heredity may exert in the progeny of seed, the results here produced do show that plants are to a very large extent influenced by their environment. Seeds grown in Kansas are quite different in chemical composition and in physical appearance from the same variety grown in another locality having different climatic conditions. That the composition of the seed has very little to do with the composition of the crop, especially when the seed has been transported to and sown in another locality having other climatic conditions, is amply shown by the data given in the tables where it is shown, *e. g.*, that the California-grown seed and the Kansas-grown seed give seed of practically the same composition when grown side by side in Kansas. It is seen that notwithstanding the fact that three plots were grown in Kansas, Texas, California, or South Dakota, from seed of the same variety possessing widely differ-

¹¹ Le Clerc and Leavitt: Seventh Internat. Congress of Applied Chemistry, Section VII, 136 (1909).

ent chemical and physical characters, the resulting crops from the three plots in each station were identical."

"Seed grown in Kansas or South Dakota shows either no starchy grains or only about 12 per cent. of such grains, yet when they are, the following year, transported to California and there grown, the per cent. of starchy grains jumps to 50 and 88 respectively. Such wheat grown in California the previous year and already somewhat acclimated gave only 40 and 71 per cent. starchy grains respectively, thus possibly showing in another way that wheats taken from a continental climate to a coast climate will show a lower value or greater deterioration the first year than seed acclimated in a coast climate. The reverse is just as true when wheat grown in a coast climate is transported to a continental climate. In this case, the California seed with 87 per cent. starchy grains gave a crop in South Dakota with only 2 per cent. starchy grains, whereas the South Dakota continuously-grown seed had 12 per cent. such grains. The California Crimean with 64 per cent. starchy grains gave a crop in Kansas with absolutely no appearance of starchy grains. It was, in fact, identical with the Kansas continuously-grown seed. These figures again show what a tremendous factor climate is. Many physical and chemical characteristics are thus influenced by the climatic conditions. These results show that the white spots on grains are not transmitted from an hereditary standpoint, nor, in fact, are any of the characteristics above mentioned. They appear, rather, to be influenced almost altogether by climatic conditions prevailing during the growing or pre-growing period. In California the weight per 1,000 and the weight per bushel are much larger than in Kansas. The per cent. of protein and the per cent. of flinty kernels are much less. The per cent. of sugar seems to increase in California over what it was or is in the Kansas and South Dakota seed, though this increase is rather small. There is not much, if any, difference in the per cent. of fat, fiber, pentosans, and ash. Similar differences are observed between California and Texas, and Kansas and Texas, though in these cases the differences are not so large. Texas is somewhat intermediate."

In these experiments the adaptation during life is apparently the important thing, though the alleged acclimatization, if established, would be a case of inheritance. These experiments, like those of Beebe on the plumage of the tanager and the bobolink, show the variations due to external conditions and seem to me to constitute a very strong argument for a more systematic and more rational study of the effect of environment during the life of the

organism with special reference to the Theorem of Le Chatelier.

In a paper read at the twenty-eighth annual meeting of the Society for the Promotion of Agricultural Science in 1907, Lyon describes some experiments in which an inheritance of acquired characters seems to have occurred to some extent. Parallel experiments were made with Iowa corn, which was grown simultaneously in Iowa and in Nebraska for two years; and seed corn from the Iowa station, planted alongside the corn which had been brought from Iowa two years before and which we may call the acclimated corn. The acclimated corn (Snowflake White) showed marked changes: the stalk had decreased almost a foot in height; the ear was nearly nine inches lower down on the stalk; the leaf area was nearly twenty per cent. less; and the date of tasselling was five days earlier. Here we get a distinct change in two years and the only question is to what extent this change is due to a survival of the fittest. Lyon says that there was no large elimination of plants through any selective process, natural or artificial; but we have no data in regard to the Iowa corn during the two years that it was becoming acclimated in Nebraska. In some experiments with wheat, he says that "the wheat seed from Iowa produced only twelve bushels of wheat the first year in Nebraska, but the small crop was due to winter killing and not to drought. The second year it produced twenty-one bushels, and the third year twenty-three bushels." I should call an elimination of half the crop a large one and for that reason I should like to know more about the corn crop before drawing final conclusions. The proper way to do these experiments would be to take the seed from the place with the more severe winter to the place with the less severe winter and not the other way round. This would eliminate the selection due to winter killing. If a fair sample of the whole crop was taken for seed each year, it ought not to be difficult to establish the existence or non-existence of the inheritance of adaptations caused by the action of the environment.

I have tried to show that the biologists have jumbled things together which do not belong together, and that they have reached erroneous conclusions in consequence of their arbitrary and unjustifiable stipulation that an acquired character shall only be considered as inherited when it breeds true for several generations after a return to the original conditions.

DR. WILEY'S RETIREMENT FROM THE BUREAU OF CHEMISTRY

Quotations from Science and the Journal of the American Medical Association¹

DR. WILEY'S OFFICIAL STATEMENT ACCOMPANYING HIS
RESIGNATION, MARCH 14, 1912

On the 9th of April, 1883, I took the oath of office and entered upon the discharge of my duties as chief of the Bureau of Chemistry, in the Department of Agriculture. For the past twenty-nine years I have endeavored to discharge these duties according to the dictates of my conscience, the knowledge at my command and the obligations of my oath. In retiring from this position after so many years of service it seems fitting that I should state briefly the causes which have led me to this step. Without going into detail respecting these causes, I desire to say that the fundamental one is that I believe I can find opportunity for better and more effective service to the work which is nearest my heart, namely, the pure food and drug propaganda, as a private citizen than I could any longer do in my late position.

In this action I do not intend in any way to reflect upon the position which has been taken by my superior officers in regard to the same problems. I accord to them the same right to act in accordance with their convictions which I claim for myself.

After a quarter of a century of constant discussion and effort, the bill regulating interstate and foreign commerce in foods and drugs was enacted into law. Almost from the very beginning of the enforcement of this act I discovered that my point of view in regard to it was fundamentally different from that of any of my superiors in office. For nearly six years there has been a growing feeling in my mind that these differences were irreconcilable, and

¹Our own comment appears in the editorial section at the end of this number, page 523.

I have been conscious of an official environment which has been essentially inhospitable.

I saw the fundamental principles of the food and drugs act, as they appeared to me, one by one paralyzed and discredited. It was the plain provision of the act and was fully understood at the time of the enactment, as stated in the law itself, that the Bureau of Chemistry was to examine all samples of suspected foods and drugs to determine whether they were adulterated or misbranded, and that if this examination disclosed such facts the matter was to be referred to the courts for decision.

Interest after interest, engaged in what the Bureau of Chemistry found to be the manufacture of misbranded or adulterated foods and drugs, made an appeal to escape appearing in court to defend their practises. Various methods were employed to secure this, many of which were successful. One by one I found that the activities pertaining to the Bureau of Chemistry were restricted and various forms of manipulated food products were withdrawn from its consideration and referred either to other bodies not contemplated by law or directly relieved from further control.

A few of the instances of this kind are well known. Among these may be mentioned the manufacture of so-called whiskey from alcohol, colors and flavors; the addition to food products of benzoic acid and its salts; of sulphurous acid and its salts; of sulphate of copper; of saccharin and of alum; the manufacture of so-called wines from pomace, chemicals and colors; the floating of oysters often in polluted waters for the purpose of making them look fatter and larger than really they are for the purpose of sale; the selling of moldy, fermented, decomposed and misbranded grains; the offering to the people of glucose under the name of "corn syrup," thus taking a name which rightfully belongs to another product made directly from Indian corn stalks.

The official toleration and validation of such practises have restricted the activities of the Bureau of Chemistry to a very narrow field. As a result of these restrictions, I have been instructed to refrain from stating in any public way my own opinion regarding the effect of these substances upon health, and this restriction has conflicted with my academic freedom of speech on matters relating directly to the public welfare.



J. M. Wiley

These restrictions culminated in the summer of 1911 with false charges of misconduct made against me by colleagues in the Department of Agriculture, which, had it not been for the prompt interference on the part of the President of the United States, to whom I am profoundly grateful, would have led to my forcible separation from the public service. After the President of the United States and a committee of Congress, as a result of investigation, had completely exonerated me from any wrongdoing in this matter, I naturally expected that those who had made these false charges against me would no longer be continued in a position which would make a repetition of such action possible. The outcome, however, has not sustained my expectations in this matter. I was still left to come into daily contact with the men who secretly plotted my destruction.

I am now convinced that the freedom which belongs to every private American citizen can be used by me more fruitfully in rallying public opinion to the support of the cause of pure food and drugs than I could with the limited activity left to me in the position which I have just vacated. I propose to devote the remainder of my life, with such ability as I may have at my command and with such opportunities as may arise, to the promotion of the principles of civic righteousness and industrial integrity which underlie the food and drugs act, in the hope that it may be administered in the interest of the people at large, instead of that of a comparatively few mercenary manufacturers and dealers.

This hope is heightened by my belief that a great majority of manufacturers and dealers in foods and drugs are heartily in sympathy with the views I have held and that these views are endorsed by an overwhelming majority of the press and the citizens of the country. In severing my official relations with the Secretary of Agriculture I take this opportunity of thanking him for the personal kindness and regard which he has shown me during his long connection with the department. I can not leave the Bureau of Chemistry without expressing to my assistants of all grades my appreciation of their loyalty and devotion to me. [Reprinted from *Science*, xxxv, pp. 498-499: March 29, 1912.]

A REPRESENTATIVE PROFESSIONAL ESTIMATE OF DR. WILEY'S
WORK AS CHIEF OF THE BUREAU OF CHEMISTRY

Dr. Wiley's service. "Damned with faint praise from the nation's chief executive, hampered by a reactionary departmental chief who has long since outlived his usefulness, badgered by a pettifogging lawyer of the night-school variety who, as a representative of the vicious interests, was able to nullify or render abortive efforts made in the interest of public health, Dr. Wiley has given up the unequal fight and handed in his resignation. Thus retires from the government service one of the most useful officials this country has ever had. An implacable foe of fraud and deceit, Dr. Wiley has for years stood between a more or less helpless public and the vested interests that have developed to a science the business of adulterating foods, sophisticating drugs and in other ways threatening the public health. On every debatable question regarding the wholesomeness of foodstuffs, Dr. Wiley was consistently on the side of the people; his superior officers have been just as persistently on the side of those who have made their millions by substituting cheap and often poisonous drugs for more expensive but wholesome foods. Assailed from without by some of the most powerful, vicious and corrupt of organizations, he was also antagonized from within by the political henchmen of the same organizations. To Dr. Wiley more than to any other one man, the public owes the Food and Drugs Act and to Dr. Wiley it also owes whatever of good has been accomplished by that act. It is to be hoped that his forced retirement will result in such an upheaval of public indignation that the forces of evil at present in control of the Department of Agriculture will be driven into political oblivion and the department filled by men who hold decency above dollars, probity above pelf and public health above private gain."

Dr. Wiley's successor. "There are two groups of people who are anxiously watching and waiting to see who will be Dr. Wiley's successor. In one group are the dishonest manufacturers, the food adulterators, the whiskey blenders, and the fraudulent patent medicine promoters: those 'interests' that are preying on the people through fraud and misrepresentation in various ways. In the other group are the people of the United States, and the honest manu-

facturers. Which of these groups will be kept in mind in the selection of the man? The newspapers announce that President Taft immediately telegraphed to the leading universities asking for suggestions as to the right man to succeed Wiley. This looks as though the President were anxious to get the right man. But the right man in this case need not necessarily be the best chemist in the country. What is needed is a man who is fundamentally honest; who has the good of the public and not of the 'interests' at heart: one, above all, who has honest convictions and has the courage to carry out such convictions. But will the President dare to ask such a man to take a position in which he will be surrounded with the restrictions that made Wiley consider it nearly useless? No self-respecting man, no man who is thoroughly qualified in every way for the position, would accept it under present conditions. There must be a further change in the personnel of the bureau which is by law presumed to enforce the Food and Drugs Act and a removal of the restrictions on its activities. Until that change is made, no one worthy to succeed Wiley ought to be expected to accept the position." [Reprinted from the editorial pages of the *Journal of the American Medical Association*, lviii, pp. 865-866: March 23, 1912.]

A SUMMARY OF THE RESULTS OF CERTAIN PHYSIOLOGICAL STUDIES ON A PEDIGREED RACE OF *PARAMÆCIUM**

LORANDE LOSS WOODRUFF

(*Sheffield Biological Laboratory, Yale University*)

(With plate 6)

The free-living Protozoa of the group Infusoria lend themselves in an admirable manner for experimental studies in general physiology. As unicellular animals they represent the organism reduced to its simplest term, and therefore many complications which arise in the study of physiology in the metazoan cell-state are greatly reduced, if not entirely eliminated. However, although research in the physiology of the Protozoa has thus far been productive of certain fundamental and far-reaching conclusions, one must confess that the results secured have not fulfilled the expectations in regard to many perplexing problems whose solution seemed comparatively easy of attainment in these lowest animal forms. This condition is not due to lack of endeavor to solve the riddles which they hold, but to an earlier inappreciation of their complexity. The wealth of literature on the Protozoa emphasizes above all the fact that, while they are the simplest, they are by no means simple animals.

The purpose of the present paper is to present a brief resumé of certain results which have been secured to the present time from physiological studies on a pedigreed race of *Paramæcium aurelia* which I have had under daily observation for more than four and one years (cf. plate VI).

This culture of *Paramæcium* was started on May 1, 1907, with a "wild" individual which was found in a laboratory aquarium. The original specimen was placed in about five drops of culture medium on a glass slide having a central ground concavity, and

* Read before the Yale Biological Club, December 7, 1911.

when this animal had produced four individuals, each of these was isolated on a separate slide to form the four lines of the culture, *Paramæcium aurelia* I. The culture has been maintained by the isolation of a specimen from each of these lines practically every day up to the present time, thus precluding the possibility of conjugation taking place between sister cells. The number of divisions of each line has been recorded at the time of isolation and the average rate of these four lines has been again averaged for varying numbers of days as the exigencies of the different experiments demanded. Permanent preparations have been preserved from time to time for the study of the cytological changes during the life history.¹ During the first eight months the culture medium consisted of infusions of hay and fresh grass, but from February, 1908, to the present time a more varied medium has been used. It was found that this race of *Paramæcium* would live in nearly any infusion of materials collected in swamps and ponds, and therefore, in an endeavor to supply as far as possible all the elements which may be encountered in the usual abode of the organism, materials were collected practically at random from ponds, hay infusions, laboratory aquaria, etc. The infusions were thoroughly boiled to prevent the contamination of the pure lines of the culture by foreign strains. This culture of *Paramæcium* has afforded an unfailing supply of free living cells for the experiments to be described. It is obvious that all the observations were made on the "same protoplasm."

I

Among the fundamental problems whose solution has been sought by numerous investigators of the Protozoa is that of protoplasmic old age, and the possible relation of conjugation to protoplasmic 'rejuvenation.' More than thirty-five years ago Bütschli and Engelmann observed that in infusorian cultures, after a number of generations, the organisms are reduced in size and show other signs of degeneration, and this afforded the first important experimental data which were advanced against the prevailing opinion that the Protozoa, because of their comparatively simple structure

¹Woodruff: *Paramæcium aurelia* and *Paramæcium caudatum*. Journ. Morphology, vol. 22, no. 2, 1911.

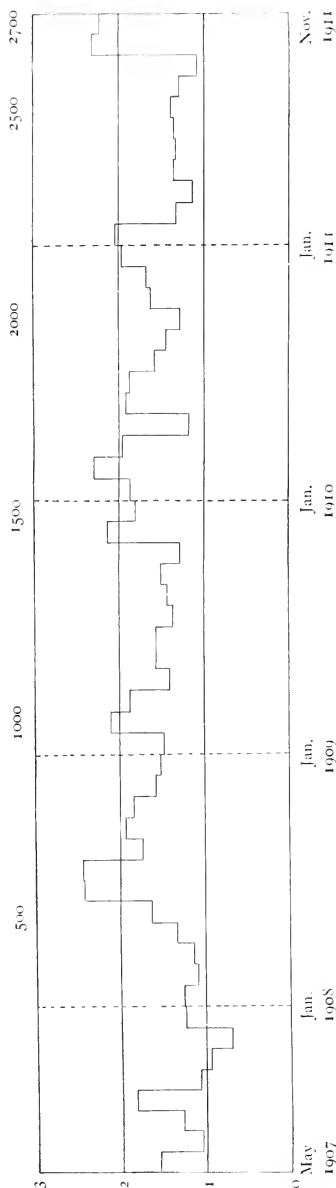
and method of reproduction, are not subject to natural death. Bütschli made the pregnant suggestion that conjugation in the Protozoa is essentially a process of Verjüngung, or restoration of the general vitality, whereby the organisms are endowed with the potential for a new period of reproductive activity, and by analogy this conception has been applied to the phenomenon of fertilization in higher forms.

A host of important studies have been made by various investigators to test the power of the Protozoa to reproduce indefinitely without conjugation, and the results secured for the most part substantiated the conclusion that only a comparatively limited number of generations may be attained by the Infusoria without conjugation or artificial stimulation.² The first point then, toward which the studies on the culture under consideration were directed, was the longevity of *Paramacium* without conjugation or artificial stimulation.

The results of the early experiments with this culture showed that the animals could not be bred for many generations in a constant culture medium of hay infusion³ and consequently a varied culture medium was used thereafter which comprised infusions of material collected and made up as already described. In culture media of this character the organisms have flourished for over four and one half years and have attained, up to the present time, 2,720 generations without conjugation or artificial stimulation. The animals are to-day in as normal morphological and physiological condition as at the start, and the rate of reproduction, which is undoubtedly the best criterion of vitality, during the past month was far above the average rate for the culture. The potential inherent, so to speak, in the original cell of this pedigreed race will be appreciated when it is recalled that theoretically the number of cells produced by this organism may be represented by 2 raised to the 2,720th power. This result, then, establishes beyond doubt the fundamental biological fact that the protoplasm of a single animal

² Cf. Calkins: *Archiv für Entwick-Mechan.*, 1902.

³ Woodruff: The life cycle of *Paramacium* when subjected to a varied environment. *Amer. Naturalist*, vol. 42, no. 500, 1908. Further studies on the life cycle of *Paramacium*. *Biol. Bulletin*, vol. 17, no. 4, 1909, p. 294. Two thousand generations of *Paramacium*. *Archiv für Protistenkunde*, Bd. 21, 1911.



WOODRUFF: PEDIGREED RACE OF PARAMOECIUM.

Graph of the rate of reproduction of the lines of the pedigreed race (I) of *Paramoecium aurelia* from start on May 1, 1907, to the present time, December 1, 1911, at the 2705th generation. The ordinates represent the average daily rate of division of the four lines of the culture, again averaged for each month of its life to date. The vertical broken lines indicate the limits of the calendar years. The figures 1,000, 1,500, etc., represent generations and are placed above the months in which they were attained.

cell may be self-sufficient to perpetuate itself indefinitely, and eliminates the phenomenon of conjugation or fertilization as a fundamental inherent necessity of protoplasm for its continued existence (cf. plate VI).

II

It being established that this race of *Paramæcium* may be bred indefinitely on a culture medium which is frequently varied, the following question suggested itself: Is the longevity on a varied environment dependent upon intrinsic stimuli from the frequent changes of the medium, or is a constant medium of hay infusion unfavorable because it lacks some elements which are essential for the continued existence of this protozoon.

To test this point it was necessary to find, if possible, a suitable constant culture medium that contains all the elements which the organism demands, and to determine its effect on the vitality of *Paramæcium* when subjected to it for a considerable length of time. If such a suitable medium could be secured on which paramæcia would live indefinitely, it is apparent that the possible continual daily stimulation afforded by varied culture media is not the crucial factor in the determination of the longevity of this culture. Further, and aside from this interesting theoretical consideration, such a favorable constant culture medium would be valuable for breeding paramæcia in many lines of experimental work, since it is well established that the reactions of paramæcia to various reagents, etc., are greatly modified by their past and present environment.

The favorable results secured first by Calkins⁴ with strong solutions of beef extract as a temporary stimulant for degenerating paramæcia cultures in infusions of hay, and later by Woodruff⁵ with *Oxytricha* under similar conditions, suggested the use of a

⁴ Calkins: loc. cit.

⁵ Woodruff: An experimental study on the life history of hypotrichous Infusoria. Journ. Exper. Zoology, vol. 2, no. 4, 1905.

weak extract of beef as a constant culture medium.⁶ Further, beef extract should afford all the elements required for the continued life of protoplasm. From a series of preliminary tests it was found that a solution of approximately 0.025 per cent. of Liebig's extract of beef gave the best results and accordingly a quantity of this solution was made up which was sufficient to provide culture medium for the organisms for a long period. The medium was put into over one hundred test tubes and these were plugged with cotton and sterilized. The solution in the various tubes remained sterile until used, and the inoculation of the medium with bacteria which were transferred with the paramæcia afforded an ample supply of food for the animals.

The regular experiment was begun by isolating from the main culture at the 2,012th generation a sub-culture which was subjected to a constant culture medium of beef extract. This sub-culture flourished on the beef extract medium until this treatment was discontinued at the end of ten months. During this period the rate of division of the "beef" culture was quite similar to that of the "varied environment" culture and neither of the cultures showed any indications of loss of vigor. The rate of division of each at the end of the experiment was practically the same as at the beginning—such fluctuations as occurred in the interval being merely rhythms. It is evident, then, that the "constant" medium employed proved to be practically as favorable a medium for the reproduction of this strain of *Paramacium* as the "varied environment" medium, and therefore the conclusion seems justified that this culture can, in all probability, be continued indefinitely on the constant medium. It therefore appears that it is the composition of the medium rather than changes in the medium which is conducive to the unlimited development of this culture without conjugation or artificial stimulation.⁷

⁶Woodruff and Baitsell: The reproduction of *Paramacium aurelia* in a "constant" culture medium of beef extract. Journ. Exper. Zoology, vol. 11, no. 1, 1911.

⁷Cf. Woodruff: Evidence on the adaptation of paramæcia to different environments. Biol. Bull., vol. 22, no. 1, 1911.

III

It is evident from many investigations on the life history of Infusoria that these organisms in pedigree cultures exhibit minor rhythmic fluctuations in the rate of reproduction. These were emphasized in a study of the life history of several hypotrichous Infusoria and defined as follows: "A rhythm is a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous."⁸

The success with beef extract as a constant medium for *Paramæcium* naturally led to an intensive study of the rhythms,⁹ in order to determine if these can be eliminated by a still more constant environment, *i. e.*, whether they are due to minor variations in the environment or to unknown intracellular phenomena, as originally stated.

The experiments in regard to the rhythms were begun by isolating sub-cultures from the main sub-culture on beef extract and placing the animals in a similar manner on depression slides in beef extract medium. The preparations were kept in chambers of a Panum thermostat at constant temperatures within the optimum temperature zone of the organism. Every precaution was taken to maintain all the conditions of the environment as constant as possible.¹⁰

A study of the rate of reproduction shows that the exceptionally and practically constant conditions of the environment failed to diminish or eliminate the rhythms—but on the contrary tended to bring them out more clearly. The fact that the rhythms appear more pronounced under the practically constant conditions existing during these experiments than they do under ordinary laboratory conditions clearly suggests that they are due to a fundamental factor in cell phenomena and not to extraneous causes. For if they are due to inherent intracellular conditions, one would a priori

⁸ Woodruff, 1905, loc. cit.

⁹ Woodruff and Baitsell: Rhythms in the reproductive activity of Infusoria. Journ. Exper. Zoology, vol. 11, no. 4, 1911.

¹⁰ For details cf. Woodruff and Baitsell, loc. cit.

expect to find them more clearly brought out when the cell is free from extraneous influences.

The division rate at the different temperatures shows that temperature, as is well known, markedly influences the rate, but it also shows that the rhythms persist—the reproductive activity being, as it were, pitched at a higher or lower scale, but its character in no wise altered.

The data just presented prove that it is not possible by constant environmental conditions to eliminate the rhythms and to resolve the graph of the multiplication rate into an approximately straight line. It therefore seems justifiable to conclude that there are inherent rhythmical changes in the phenomena of the cell which are brought to view still more clearly when not influenced by external factors.

Finally, the data justify the conclusion that the cells of this pedigreed race of *Paramæcium aurelia* have the potentiality to perpetuate themselves indefinitely by division (under proper environmental conditions)—the only necessary variations in the rate of reproduction being normal minor periodic rises and falls of the fission rate, due to some unknown factor in cell phenomena.

IV

Considerable data have been accumulated in regard to the effect of different temperatures on living processes in general, and on the rate of reproduction of Infusoria, but comparatively little work has been undertaken with special reference to the temperature coefficient. Vital phenomena are undoubtedly largely the result of chemical reactions taking place in the living substance, and consequently one would expect that the well-known law of van't Hoff and Arrhenius should apply, *i. e.*, for each increase of 10° C. the rapidity of a reaction is increased from two to three times. It was therefore decided to make a detailed study of the effect of several constant temperatures on the rapidity of cell division of this culture of *Paramæcium* when subjected to a 'constant environment' of beef extract.¹¹

¹¹ Woodruff and Baitsell: The temperature coefficient of the rate of reproduction of *Paramæcium aurelia*. Amer. Journ. Physiology, vol. 29, no. 2, 1911.

It has just been shown that there are normal rhythmic fluctuations in the rate of reproduction of *Paramacium*, even when subjected to the most constant environmental conditions, and that the rhythms are due to some unknown inherent factor in cell phenomena. The rhythms, then, are independent of temperature. Records of a line of cells with the descending phase of a rhythm predominant, subjected to 28° , and a line of cells with the ascending phase of a rhythm predominant, subjected to 24.5° , may actually show (during the persistence of the rhythm) a more rapid rate of division at the lower than at the higher temperature. Accordingly, in this study it has been necessary to be sure that the animals subjected to the different temperatures were in comparable phases of the rhythm, or that the experiments were sufficiently prolonged to include one or more complete rhythms. It is clear that the rhythms are a factor which must be taken into account in any study of the physiology of this animal.

The first point to determine was the general effect of various temperatures on the protoplasm of this culture, and accordingly subcultures, comprising eight lines of "sister" cells, were subjected to the following temperatures for forty days or until the temperature proved fatal: 8° , 16° , 21° , 24.5° , 28° , and 32° C. The results were clean cut and showed that the organisms are adapted to a constant temperature below 32° and above 21° C. A careful study of the daily records indicates that the optimum temperature zone is between 24° and 28.5° .

Having determined the optimum temperature zone, the temperature coefficients for extreme points within this zone were studied. Eight lines of cells at 28° for 40 days gave a total of 527 divisions, and eight lines of cells at 24.5° for 40 days gave a total of 370 divisions. Employing the extra- and interpolation logarithmic formula frequently used for such computations, which takes the form

$$Q_{10} = \left(\frac{K_1}{K_0} \right)^{\frac{10}{T_1 - T_0}}$$

in which Q_{10} is the coefficient of the increase in reaction velocity for a rise of 10° C., and the symbols K_1 and K_0 represent constants

observed (in this case cell divisions) at the temperatures T_1 and T_0 respectively, stated in degrees Celsius, it is found that the temperature coefficient derived from the above experiment is 2.74. Again, if the coefficient is computed for the rate of division of, for example, lines 1, 2, 3, and 4 (of the eight lines) at 28° , and of lines 1, 2, 3, and 4 (of the eight lines) at 24.5° , it is found to be 2.71. Likewise Q_{10} for only line 1 is 3.04. Obviously these coefficients are in remarkable agreement with the demands of van't Hoff's law.

In the experiments described, a comparatively limited number of lines of cells were tested for a considerable period of time at temperatures within the optimum zone of the organisms. It seemed advisable also to determine the effects of different temperatures on a large number of lines of cells for a shorter period of time. This not only afforded, in a sense, a reciprocal experiment, but also allowed the organisms to be tested at temperatures 7° apart instead of only 3.5° apart, because for a short period, as five days, a normal rate of division could be obtained at 21° . Accordingly 100 lines of cells were subjected to 28° C. for five days and a similar number to 21° C. for five days. The 100 lines at 28° gave a total of 662 divisions, while the 100 lines at 21° gave a total of 336 divisions. Computation of Q_{10} from these data by the formula previously employed gives 2.63, which again is clearly in remarkable agreement with van't Hoff's rule.

In order to test the coefficient at a lower range of temperature, three series of eight lines of cells each were carried for five days at 28° , 24.5° and 16° C. respectively. It was necessary to make the period short because the organisms were not long able to endure 16° , and beyond five days the rate of division was obviously abnormal because the cells showed a high mortality. The records of this experiment show that 71 divisions occurred in the eight lines at 28° for five days, 44 divisions at 24.5° , and 18 divisions at 16° . Q_{10} for the division rates at 28° and 16° is 3.13, and at 24.5° and 16° is 2.86. Again both of the coefficients conform closely to theoretical demands, and the fact that they are slightly higher than the average coefficient for the data given above is in accord with the results of experiments on other physiological processes—it being

quite well established that the rate of acceleration decreases as one proceeds upward from 0° toward the optimum temperature.

The average of the coefficients of the four experiments is 2.84. If, however, the coefficients are weighted on the basis of the rate of division of one cell for five days, the most fair coefficient for the entire series of experiments is obtained, $Q_{10} = 2.70$. The obvious conclusion is that the rate of division of *Paramacium* is influenced by temperature at a velocity similar to that for a chemical reaction.

V

The recent developments of the ionic theory have lent renewed interest to the problem of the physiological action of salts upon protoplasm and have brought forward strong evidence to support the idea that the pharmacological action of a salt solution is to a considerable extent due to the ions into which the salt dissociates. Mathews has elaborated and emphasized the idea that atoms act by means of their electrical condition, and that positively and negatively charged ions have opposite action. Ions of the same sign act alike, but the degree of their action, *i. e.*, their specific toxicity, differs because the ease with which they change their electrical condition varies. The poisonous action of an element, then, depends to a considerable extent upon the affinity of the atom for its electrical charge. Mathews has suggested the term "ionic potential" to indicate the tendency of any ion or atom to change its electrical state, *i. e.*, the inherent tendency of any ion in any concentration to change into an atom of its metal.

Comparatively few experiments have been performed on animal organisms to determine if toxicity bears any direct relation to ionic potential and therefore it seemed worth while to determine the relative toxicity of a number of cations toward the protoplasm of *Paramacium*.¹²

The experiments were planned to determine the concentration of any particular salt necessary to kill within two seconds one half

¹² Woodruff and Bunzel: The relative toxicity of various salts and acids toward *Paramacium*. Amer. Journ. Physiology, vol. 25, no. 4, 1909.

of the organisms tested, at about 20° C. The criterion of death was the stopping of the cilia and the consequent loss of motion of the organism. It was possible to distinguish this point with great exactness owing to long familiarity in handling paramæcia in pedigreed culture work. To ascertain the exactness of the method, the toxicity of the salt was determined on successive days, and the agreement of the results proved the conditions of the experiment to be highly satisfactory. In certain cases over 100 determinations were made before the desired toxicity was secured.

The salts and acids employed were: AgNO_3 , HgCl_2 , CuCl_2 , FeCl_3 , PbCl_2 , NiCl_2 , HCl , H_2SO_4 , CoCl_2 , CdCl_2 , ZnSO_4 , MnCl_2 , MgCl_2 , SrCl_2 , CaCl_2 , and KCl . In almost every case the chloride of the metal was used, but the fact that a sulphate and a nitrate were employed does not render the data less comparable, because the ionic potential of the three anions is very nearly the same. Moreover, Mathews has shown that the same concentration of the nitrate, sulphate and chloride of sodium are required to inhibit the development of the eggs of *Fundulus*. This is also shown by the toxicity of sulphuric and hydrochloric acid as found in these experiments on paramæcia. (For details, see original paper.)

The data secured clearly indicate a parallelism between the smallest fatal concentration of the various cations and the ease with which they throw off their charge, *i. e.*, their ionic potential. As in the results of previous workers, there are certain metals which do not appear in the order of toxicity which would be expected from their potential. However, one must consider that the living cell is composed of a large variety of materials, each having specific affinities for the different ions, and it is probable that the low toxicity of copper, for example, is based on differences of this sort. Cadmium and ferric iron are also out of place, just as they have been found to be in their action on the eggs of *Fundulus* and certain seedlings. Hydrogen is somewhat more toxic than one would expect on first thought, but this is probably due to the high migration velocity of the hydrogen ions.

Apart from these few exceptions all of the cations tested follow the order of their ionic potentials, the slight fluctuations being within the errors of the experiment and may not be considered as excep-

tions. Considered as a whole, the results of the experiments indicate a marked parallelism between the order of toxicity of the various cations toward *Paramacium* and the ionic potentials of the ions employed.

VI

Hunt's interesting experiments on the effect of small doses of alcohol on mice and guinea pigs apparently indicated that animals to which this substance has been administered for some time possess increased susceptibility to certain poisons. It was from this point of view that a series of experiments were undertaken on this culture of *Paramacium*.¹³ Experiments were planned to determine the influence of small doses of alcohol on the vitality of the culture as indicated by the rate of multiplication; and also to determine if the animals showed increased susceptibility to copper sulphate. It is impracticable to present the details of the results secured by subjecting various subcultures to alcohol alone and to alcohol and copper sulphate simultaneously, but it is believed that the data secured warrant the following statements:

Minute doses of alcohol (*e. g.*, 1/2,500 to 4/2,500) will decrease the division rate at one period and increase it at another period of the life of the culture. When alcohol increases the rate of division, the effect is not long continued, but gradually diminishes and finally the rate of division falls below that of the control, followed by fluctuations above and below the rate of the control. An increase (doubling) of the amount of alcohol administered, however, will again bring about more rapid cell division for a limited period. The amount of alcohol has been increased (doubled) three times, always with the same result. Treatment with alcohol lowers the resistance of the organisms to copper sulphate (*e. g.*, 1/1,250,000 CuSO_4).

It is of considerable interest that alcohol produces opposite effects on the division rate at different periods, and this shows the danger of drawing conclusions from experiments on short-period cultures or on individuals about the ancestry of which little or nothing is known and which have been isolated merely from stock cul-

¹³ Woodruff: Effects of alcohol on the life history of Infusoria. Biological Bulletin, vol. 15, no. 2, 1908.

tures. The same point is illustrated by some previously published experiments with the salts of potassium in which it was found that the dibasic potassium phosphate caused an acceleration of the rate of division during one part and a retardation of the rate during another part of the life of the culture.

The data show that organisms which have been subjected for long periods to small amounts of alcohol, and which have attained a greater number of generations than the non-alcohol series, are more susceptible to copper sulphate; and that the organisms which have been subjected to the greater strength are more susceptible to copper sulphate than those subjected to the less strength. This shows clearly that alcohol, in the small amounts which may be said to be "beneficial" from the standpoint of metabolism, since more cell divisions have occurred, definitely renders the cells more susceptible to the "injurious" effects of copper sulphate. In what way this is brought about is not evident from the results secured to date. It seems improbable that we are justified in assuming that the alcohol has brought about a general "lowering of resistance," in view of the fact that the general effect of the alcohol is to increase cell division. The results suggest that probably alcohol effects some change in the permeability of the cell membrane to copper sulphate.

VII

Evidence from many sources points to the conclusion that excretion products, in the case of many organisms, have a profound effect on cell division and growth, and also that the Infusoria, under favorable conditions of food and temperature, excrete considerable amounts of carbon dioxide, together with various other end-products of metabolism, which may reasonably be expected to be evident through biological as well as chemical tests.

The ordinary hay infusion, teeming with animal and plant life, is a microcosm in which every organism may, and probably does, in some degree affect the well-being of every other organism present. Besides the obvious influence exerted by animals in feeding on other forms and by green plants through photosynthetic processes, one would expect the effects of organisms on their environment by the elimination of products of their metabolism or

excretion products to be one of the most important. The interdependence of the organisms of a hay infusion is so complex that, taken as a whole, it is almost beyond the possibility of analysis, and therefore the logical method of approach to the subject is to study the interactions of isolated organisms and small groups of organisms on themselves and on each other.

Accordingly there have been made the initial experiments of a series which is planned to elucidate, if possible, some of the complex factors at work in a hay infusion; for example, those which determine the interdependence of the organisms, their sequence, time of appearance, disappearance, etc.¹⁴ The data outlined were derived from the study of the effect of different volumes of culture medium on the rate of reproduction of *Paramœcium*; the effect of changing the culture medium at twenty-four and forty-eight hour intervals on the rate of reproduction; and the effect of culture medium, in which many paramœcia have been living, on the rate of reproduction.

In the series of experiments which were made to determine the effect of different volumes of culture medium, the volumes selected were two, five, twenty and forty drops. In brief, the daily rate of the organisms in two, five, twenty, and forty drops of culture medium changed every twenty-four hours showed that, for example, those in five drops divided 2.4 per cent. more rapidly than those in two drops, those in twenty drops divided 6.4 per cent. more rapidly than those in two drops, and those in forty drops divided 7.4 per cent. more rapidly than those in two drops. It is believed that the experiments were sufficiently comprehensive to establish clearly the fact that the rate of reproduction of paramœcia is influenced by the volume of the culture medium (within the limits tested in the experiments), and that the greater the volume the more rapid is the rate of division.

It being clear that in an increased volume of culture medium there is increased division rate, the next point of importance was to determine to what factor or factors this is due. It is evident that it may be the result of variations in temperature, pressure,

¹⁴ Woodruff: Effect of excretion products of *Paramœcium* on its rate of reproduction. Journ. Exper. Zoology, vol. 10, no. 4, 1911.

surface of medium exposed to atmosphere, food supply, excretion products of bacteria or excretion products of paramæcia. It will suffice to state that each of these factors was carefully weighed and the conclusion was reached that the variation in the daily division rate in the different volumes of water is due to the excretion products of the paramæcia themselves.

If this conclusion is correct, the effects of the excretion products should manifest themselves more clearly in cultures in which the organisms remained in the medium a longer period, than in those in which the organisms remained in the medium a shorter period of time. To test this a second series of experiments was carried on simultaneously with those already described, and in this second series the animals remained in the same medium for forty-eight hours instead of twenty-four hours. The culture in which the medium was changed at forty-eight hour intervals showed that the organisms in a volume of five drops divided over 5 per cent. more rapidly than those in two drops; and that those in twenty and forty drops divided over 9 per cent. more rapidly than those in two drops.

The results, then, of this series of cultures in which the organisms were isolated every forty-eight hours, confirm the general conclusion derived from the series isolated every twenty-four hours, *i. e.*, in general an increased volume of medium is conducive to more rapid multiplication, and further it clearly shows that the gain in division rate in the forty-eight hour series in five, twenty, and forty drops over that in two drops is in every case greater than the gain of the twenty-four hour series in five, twenty, and forty drops over that in two drops. Again, from a consideration of the data of comparable cultures changed daily and that of cultures of equal volumes of media changed on alternate days, it is found that the gain of the series changed daily over those changed at forty-eight hour intervals is over eight per cent. in the case of two drops, and slightly over six per cent. in the case of five, twenty and forty drops. Consequently, as one would expect, changing the medium on alternate days has most influence in the smallest volume of medium.

It is clear, then, that all the data derived from the experiments

mentioned point to the conclusion that paramæcia excrete substances which are toxic to themselves and that these substances, as one would expect, are more effective when the organisms are confined in limited volumes of culture medium.

The logical method of procedure was to determine the influence of media known to be contaminated with the excretion products of large numbers of paramæcia. A series of experiments were accordingly made with media which, it is believed, were identical except that one contained the products of metabolism of a heavy growth of paramæcia, while the other was absolutely free from such contamination. The lines of organisms subjected to the medium which had previously been the abode of numerous paramæcia reproduced much more slowly than those in the uncontaminated medium, so it is obvious that culture media in which paramæcia have been living have a decidedly depressing effect on the rate of reproduction of *Paramæcium*. All the results indicate that the excretion products of Protozoa play an appreciable part in determining the period of maximum numbers, rate of decline, etc., of the fauna of hay infusions.¹⁵

SUMMARY

The chief points which, I believe, the studies on the physiology of this race of *Paramæcium* have shown are:

1. The protoplasm of a single cell has the potentiality to reproduce by division indefinitely—under favorable environmental conditions—and therefore conjugation, or fertilization, is not a necessary phenomenon for the continued life of protoplasm.
2. The more or less general opinion that a constant environment is detrimental to breeding stock and results in decreased activity, degeneration and sometimes death, is not substantiated by the results from this culture on a constant environment of beef extract. The fact that these experiments were on the protoplasm of one of the simplest animals, indicates, I believe, that the prevailing opinion is based on observations which include incidental complicating factors not recognized.

¹⁵ Woodruff: Observations on the origin and sequence of the protozoan fauna of hay infusions. Journ. Exper. Zoology, vol. 12, no. 2, 1912. Fine: Chemical properties of hay infusions, with special reference to the titratable acidity and its relation to the protozoan sequence. Ibid.

3. There are normal minor rhythms of the rate of reproduction of *Paramacium* which are due to unknown intracellular phenomena.

4. The temperature coefficient of the average rate of multiplication is approximately 2.70, and consequently the rate is influenced by temperature at a velocity similar to that for a chemical reaction.

5. There is a marked parallelism between the order of the toxicity of many cations toward *Paramacium* and the ionic potentials of the ions.

6. Subjection to small amounts of alcohol increases the susceptibility of the organisms to copper sulphate.

7. Excretion products of *Paramacium* have a decidedly depressing effect on its rate of reproduction.

BIOCHEMICAL STUDIES ON SOILS SUBJECTED TO DRY HEAT

FRED J. SEAVER AND ERNEST D. CLARK

(Laboratories of the New York Botanical Garden and of the Department of Biological Chemistry of Columbia University)

(With plate 7)

INTRODUCTION

In our earlier communications on the relation of heated soils to the growth of plants we concerned ourselves only with the effects on the fungi, especially *Pyronema*. In our first paper¹ we reported that this fungus fails to develop on unheated soils, but can be made to thrive and develop abundant fruit on soils that have been heated. At that time it was suggested that heat seemed to produce chemical changes in the soil as well as to eliminate the competition of bacteria, other fungi, etc., with *Pyronema*. In the second paper² our experiments indicated that the inhibiting factor in unheated soils did not seem to be a toxin destroyed by heat, as had been claimed by Kasaroff. On the contrary, the better growth on soils which had been heated in an oven appeared to be parallel to the amount of soluble matter and to the depth of color of the water extract, both of which in turn were dependent upon the temperature to which the soils had been exposed. Temperatures of 125° to 180° C. gave very dark-colored extracts, with the odor of burnt sugar, due to the large amount of peculiar organic substances held in solution. Extracts obtained from such heated soils proved to be ideal culture media for *Pyronema* and others of the lower fungi, judging from the difficulty we had in keeping the extracts sterile. The addition of heated soil extracts to unheated soil did not render it favorable for *Pyronema* growth; and furthermore, analysis showed that

¹ Seaver: Studies on pyrophilous fungi. I. The occurrence and cultivation of *Pyronema*. *Mycologia*, 1: 131-139. 1909.

² Seaver and Clark: Studies on pyrophilous fungi. II. Changes brought about by the heating of soils and their relation to the growth of *Pyronema* and other fungi. *Mycologia*, 2: 109-124. 1910.

the unheated soil removed nearly all the soluble matter in the deeply colored extracts allowed to percolate through it. This interesting phenomenon has been noticed by us repeatedly, but whether it is due to chemical, physical or physico-chemical action we can not say. Since we first became interested in the effects produced by heating soil several other investigators have published the results of their studies on this subject. We have confined ourselves to the use of dry heat, while nearly all the others used steam heat.

DISCUSSION OF THE WORK OF OTHER INVESTIGATORS

At the Rothamsted Experiment Station in England, Russell and Hutchinson³ studied the effects on soils produced by heating to a low temperature (98° C.) and by treatment with volatile antiseptics. Under these conditions they found that the fertility of the soil was enhanced and that this was due to an increase in the nitrogenous food thus made available. This effect seemed to be the result of the quickened activities of certain nitrifying bacteria not present to so great an extent in unheated soils. These authors' experiments show that the inhibition of the beneficial organisms in untreated soil is not due to a toxic substance, but that it is caused by the presence in untreated soils of large protozoan organisms which destroy the useful bacteria. Heat and antiseptics kill the protozoa and most of the bacteria, but the latter soon take a new lease of life from the unharmed spores, and finding themselves unattacked by their enemies, they reproduce in great numbers, meanwhile causing large increases in the soluble nitrogenous matter of treated soils. Experiments with crops demonstrated that the higher plants made growths on treated soils which were much better than on soils not treated. In summing up Russell and Hutchinson's work we may say that their idea is to credit the increased productiveness of heated and toluened soils to the consequent destruction of protozoa which in ordinary soils prey upon the beneficial ammonifying bacteria and nearly exterminate them.

Lyon and Bizzell⁴ at the Cornell Experiment Station published

³ Russell and Hutchinson: The effect of partial sterilization of soils on the production of plant food. *Jour. Agric. Sci.*, 3: 111-144. 1909.

⁴ Lyon and Bizzell: Effect of steam sterilization on the water-soluble matter in soils. *Bull.* 275. Cornell Experiment Station, Ithaca, N. Y. 1910.

a bulletin on the effects of steam sterilization, studying the chemical and agricultural phases rather than the bacteriological ones. Their soils were steamed for various periods of time in an autoclave under a pressure of two atmospheres. The water-soluble matter was greatly increased by this treatment, but upon standing untouched the soils gradually went back to their original state, at least as far as their soluble matter was concerned. These authors found that both ammonification and nitrification were absent for three months after heating. The extracts of these steamed soils were distinctly unfavorable to the growth of seedlings, but upon proper dilution the seedlings made a better growth than in extracts of unsteamed soils. They speak of the "injurious" results of steaming, but, of course, this term applies only when judged from the standpoint of the green plant. The lower fungi like *Pyronema* flourish on these extracts which are toxic to the seedlings of the higher plants. After standing three months the steamed soils made an excellent showing when planted with wheat. The fact that ammonification is at a standstill, and that such steamed soils are harmful to green plants immediately after this treatment, is probably attributable to the same cause, namely, the complete destruction of beneficial bacteria by the higher temperatures they employed. Russell and Hutchinson did not find this to be the case, probably because the temperature they used was sufficient to kill protozoa but not the spores of bacteria. This variation in temperature might well account for the differences in the results of these two groups of investigators. However, it is doubtful if it is correct to speak of the sterilization of a soil, since, as pointed out to us by Dr. Schreiner of the Bureau of Soils, it is impossible to say that the heat has entered the interior of the soil mass sufficiently to kill every living organism and spore. Fletcher⁵ does not believe that the advantage of heating soils comes from changing the bacterial flora or increasing the soluble matter. He feels that the heat destroys some toxin present in the unheated soil. He heated soils to 95° and 170° C. and found that afterwards corn made a better growth upon both the heated soils than upon the unheated control. The work of other investigators and also our own does not agree with Fletcher's conclusion that soils heated to

⁵ Fletcher: Effect of previous heating of the soil on the growth of plants and the germination of seeds. *Cairo Science Jour.*, 4: 81-6. 1911.

a high temperature are favorable to an immediate growth of green plants.

Bolley⁶ concludes that the destruction of the spores of disease-producing fungi and bacteria has more to do with the final increased productiveness on heated soils than has either the destruction of bacteria-loving protozoa, as claimed by Russell and Hutchinson, or the increase of soluble matter found by Lyon and Bizzell. There is undoubtedly considerable truth in this contention of Bolley. The authors of the present paper feel that the whole question of the effects of heating soils is a very complex one and one in which the experimenter's interpretation of results depends upon his training and point of view; whether it be bacteriological, chemical or phytopathological. It is very likely that the truth of the matter lies somewhere on the border-lines of the three sciences indicated.

PRACTICAL ASPECTS OF SOIL "STERILIZATION"

For some time a few gardeners and florists have found that heating the soil for the benches of their greenhouses seems to produce better plants, which were also free from the attacks of disease and from competition with weeds. In India, also, the Hindu farmers have long been in the habit of burning brush, cow-dung, etc., upon the surface of the rice seed-beds. They call this process "rab" and all speak highly of its efficacy in producing good crops. Mann⁷ has discussed this practice and believes there are three main causes for its beneficial results: (1) Favorable changes in the bacterial flora of the soil; (2) organic nitrogenous matter is made more soluble; (3) the physical properties of the soil seem to be improved. Until within a few years the practice of heating soils in agricultural work had but few advocates; but now through the influence of the experiment stations and government bureaus it is being extended and applied in many directions.

Recently various bulletins and circulars have been published in different states and countries, advocating the "sterilization" of soils by heat, thereby freeing the crops from fungus and insect pests and also killing the seeds of weed plants. This treatment has been

⁶ Bolley: Interpretation of results in experiments upon cereal cropping methods after soil sterilization. *Science*, 33: 229-34. 1911.

⁷ Mann: Ann. Report of Dep't. of Agriculture, Bombay, pp. 50-4. 1908-9.

especially recommended in the case of seed-beds prepared for starting tobacco plants. Portable boilers with inverted pans and other apparatus for sterilizing the soil with steam have been described, and the results discussed in publications from the Connecticut Agricultural Experiment Station⁸ and from the Bureau of Plant Industry at Washington.⁹ In a bulletin¹⁰ from South Africa the advantages of soil sterilization are called to the attention of the tobacco growers, the increase of plant food being especially noted. Burning brush or similar material upon the soil seems to yield better results than the use of steam. Now, in all these reports upon the favorable results of heating soils we find little reference made to any effects except the desirable ones of killing the spores of parasitic fungi, etc. The chemical changes that may be produced are almost wholly ignored, yet, even under the condition of the rather low heat obtained with the steaming apparatus, such changes apparently have a stimulative and beneficial action on the plants. Furthermore, we believe the almost universal practice of "sterilizing" the soil to be used in physiological and culture experiments should be applied with caution, and with due recognition that the resultant chemical changes in such soils may vitiate experimental results and prove more disconcerting than the undesirable factors in untreated soils.

Observers in certain districts in Maine and other parts of the country where blueberries flourish over large areas, have often noticed that when such areas have been recently burned over the growth was especially luxuriant. The Indians also noticed this phenomenon and so they occasionally set fire to large tracts of blueberry lands in order to encourage large crops of fruit in the following two or three summers. In northern New England, blueberries are picked in large quantities for canning, and in these districts the owners of blueberry pastures burn over one-third of such land every third year, thus burning over the whole once every three years.¹¹ The

⁸ Hinson and Jenkins: The management of tobacco seed-beds. Bull. 166, Conn. Agric. Experiment Station, New Haven. 1910.

⁹ Gilbert: The root-rot of tobacco caused by *Thielavia basicola*. Bull. 158, Bur. Plant Industry, Dep't of Agriculture, Washington. 1909.

¹⁰ Scherffius: Sterilizing tobacco seed-beds. Agric. Jour. of Union of South Africa, 2: 418-31. 1911.

¹¹ Munson: The horticultural status of the genus *Vaccinium*. Bull. 76, Maine Agricultural Experiment Station, Orono. 1901.

attractiveness of the blueberry has often led to attempts to transplant and grow it under artificial conditions but failure usually followed. This was explained by assuming that the blueberry loved a peaty soil, a condition which could not be successfully imitated in gardens. However, Coville¹² has recently succeeded in growing the blueberry from seed and bringing it to full size and maturity. This was not done until a careful field study had shown that in nature this plant flourishes upon peaty soils which are *acid* in character. With this fact in mind Coville used well-drained acid soils, and by so doing he had unusual success in raising the blueberry. In the roots of the blueberry plant he discovered a mycorrhizal fungus that seemed to help supply the plant with nitrogenous food. In acid soils the ordinary nitrogen bacteria cannot develop, and this type of fungus may take their place in plants like the blueberry and the cranberry which thrive on acid peaty soils. In a later note Coville¹³ reported that the Mayflower, or Trailing Arbutus, could be raised from seed when sown upon such acid soils, and could then be brought to a perfection of bloom seldom equalled in the wild state. We feel that there is some connection between these two observations that certain plants flourish upon acid soils and that many of the same kinds flourish on burned-over soils. In our earlier work we found that heating a soil produces in it easily soluble substances of acid reaction. It seems likely that burning over the underbrush on a soil renders it acid in reaction, and probably produces an artificially acid condition nearly as agreeable to certain plants as are the naturally acid peat soils. To test this point we now have experiments under way with seeds and cuttings of the blueberry, and also with the seeds of the Mayflower planted in soils rendered acid by heating.

ANALYTIC CHEMICAL STUDIES

In our previous paper the data for composition of the extracts of soils exposed to dry heat show that the amounts of soluble matter in the extracts are from six to ten times greater than in similar

¹² Coville: Experiments in blueberry culture. Bull. 193, Bur. of Plant Industry, United States Dep't of Agriculture, Washington. 1911.

¹³ Coville: The use of acid soil for raising seedlings of the Mayflower, *Epigaea repens*. Science, 33: 711-2. 1911.

extracts of unheated soils. The organic matter thus made soluble is always greater in amount than the inorganic matter, but the latter is also considerably increased. We now turned our attention to a study of the effect of exposing the soil to different intensities of heat for the same period of time. The manner of handling the soil for these experiments was as follows: Ordinary loamy soil from this vicinity was placed in pots properly labeled with the various temperatures to which they were to be heated. They were all placed in a large hot-air sterilizing oven and were heated until the thermometer in the soil indicated 90°. This temperature was maintained for two hours, when the pot marked 90° was removed. The temperature was then increased to 120°, and maintained there for two hours, when the corresponding pot was taken out. This process was repeated in each case until the different series of heated soils were obtained. The extracts of the various soils were then prepared by percolating 3 kg. samples of the soils with about two liters of distilled water. We saved the first 350 c.c. that came through and used two 50 c.c. portions for the determination of soluble matter and total nitrogen. The remainder of each extract was reserved for cultural experiments with seedlings, etc.

TABLE 1.

Effect of Different Temperatures upon the Soluble Matter in Soils

Nature of Soil	Total Solid Matter, Per Cent	Organic Matter, Per Cent	Inorganic Matter, Per Cent	Total Nitrogen, Per Cent
Unheated.....	0.030	0.020	0.010	0.0012
Heated to 90°.....	0.061	0.045	0.016	0.0016
Heated to 120°.....	0.117	0.092	0.025	0.0036
Heated to 150°.....	0.219	0.177	0.042	0.0092
Heated to 170°.....	0.275	0.184	0.091	0.0112

TABLE 2.

Conditions Similar to Those Stated in the Heading of Table 1

Nature of Soil	Total Solid Matter, Per Cent	Organic Matter, Per Cent	Inorganic Matter, Per Cent	Total Nitrogen, Per Cent
Unheated.....	0.030	0.015	0.015	0.0017
Heated to 90°.....	0.033	0.019	0.014	0.0022
Heated to 120°.....	0.049	0.034	0.015	0.0084
Heated to 170°.....	0.111	0.069	0.042	0.0106

To determine the soluble matter the 50 c.c. samples of each extract were evaporated to dryness in platinum dishes, dried at 108° to constant weight and this weight recorded as *total solids*. The residues were carefully ashed at a low red heat, dried and weighed again, and this weight recorded as *inorganic matter*. The difference between this weight and the weight of the total solids was recorded as *organic matter*. Total nitrogen was determined in another 50 c.c. portion by the official method for nitrogen, including that in the form of nitrates, etc.¹⁴ The analytical results were very interesting. We give two typical series of such data in Tables 1 and 2.

From such figures one may conclude, as was done in our previous paper and in the papers of others, that dry heat causes the production in soils of large amounts of soluble material. The more striking fact is that the *increase of soluble matter goes hand in hand with the temperature to which the soil was subjected*. It occurred to us that the objection might be raised that in our experiments it was the *duration* of the heating rather than the temperature that caused the gradual increase of soluble matter in every one of our different series. To test this point we exposed one lot of soil to 120° for ten hours, and upon analysis of the extract it showed only a slight increase of soluble matter over the lot heated to 120° for two hours. The intensity of the heat seems to be the controlling factor in rendering the soil material more soluble. The organic matter, inorganic matter and total nitrogen all seem to be affected by the heat, but the organic matter and nitrogen show the greatest and most consistent increases. The color, odor and degree of contamination with *Pyronema*, *Penicillium*, etc., all show an increase parallel to the soluble matter indicated by analysis. In the next section our culture experiments show the same gradual change in the properties of the heated soil extracts dependent upon the degree of heating.

CHEMICAL NATURE OF THE SOLUBLE ORGANIC MATTER IN HEATED SOILS

We have already reported that the extracts of heated soils are acid to litmus, give the Molisch test for carbohydrates, reduce Fehling solution, and give a precipitate with lead acetate solutions.

¹⁴ Official and provisional methods of analysis. Bull. 107 (rev.), Bur. of Chemistry, U. S. Dep't of Agriculture, Washington, 1908, p. 7, sec. (c).

This was the extent of our knowledge when we began the present study. Our first experiments were performed upon 200 c.c. of heated soil extract containing about 0.3 per cent. of total soluble matter. Its color was deep brown, its acidity was marked and it had an odor of burned sugar. To this hot extract an excess of basic lead acetate solution was added; this caused a heavy brown precipitate, which was filtered off and washed. The dark precipitate was suspended in distilled water and treated with hydrogen sulfid gas for the removal of the lead. The lead sulfid thus formed was filtered off and the clear brown filtrate was boiled to expel the excess of hydrogen sulfid. The filtrate after the removal of the first lead precipitate was yellowish and it, too, was saturated with hydrogen sulfid to remove the excess of lead. The lead sulfid was removed by filtration. The two solutions now free from both lead and hydrogen sulfid were then subjected to the tests indicated below:

	Molisch Test	Fehling-Benedict Solution	Alkaline Silver Solution	Color	Odor
Material <i>precipitated</i> by basic lead acetate...	Very strong	Reduction	Reduction	Brown	Sharp, caramel-like
Material <i>not precipitated</i> by basic lead acetate.....	Doubtful	Negative	Reduction	Yellowish	Sweet, not sharp

The solution of the material precipitated by basic lead acetate deposited a crystalline powder upon spontaneous evaporation to dryness. These crystals were insoluble in 95 per cent. alcohol, which dissolved the brownish coloring matter mixed with them. This treatment might afford a means of separating these substances. Dialysis through collodion membranes did not produce any separations in any of our chemical work upon any of the various soil extracts.

We next obtained a large volume of heated soil extract (2.25 l.) and added to it *normal* lead acetate solution until no more precipitate was produced. This precipitate was filtered off and *basic* lead acetate solution added to the filtrate until no further effect was produced. In this case the addition of the basic salt caused a precipi-

tate which became lighter in color with each successive addition. This precipitate was removed by filtration and the clear yellow filtrate was saturated while hot with hydrogen sulfid. This was also done with suspensions of each of the two precipitates already obtained. The lead was removed as sulfid and the excess of hydrogen sulfid was boiled out. The three purified solutions were tested as follows, with the results indicated:

	Molisch Test	Fehling-Benedict Solution	Alkaline Silver Solution	Color	Odor
Material precipitated by <i>normal</i> lead acetate...	Weak	Reduction (?)	Reduction	Brown	Sharp
Material precipitated by <i>basic</i> lead acetate...	Very strong	Reduction	Reduction	Brown	Sweet
Material precipitated by <i>neither</i> reagent.	Very strong	Reduction (?)	Heavy precipitate	Light yellow	Sweet

Note. An attempt was made to prepare osazones from the solutions by the customary use of phenyl hydrazine and sodium acetate but dark amorphous products were always obtained.

The three solutions obtained, as described in the last paragraph, from the basic lead acetate precipitate, the normal lead acetate precipitate, and the final filtrate, were all evaporated to small bulk. With these solutions various attempts to make separations were ineffectual until we found that the addition of an alcohol-ether mixture or acetone produced light flocculent precipitates. Acetone yielding better results, we used it upon larger volumes of the three solutions. The acetone produced buff-colored precipitates of great bulk but containing little material when dry. They dissolved easily in distilled water. Such solutions showed little if any reduction of Fehling-Benedict solution, but gave strong Molisch tests in all cases. They gave reddish brown colorations with dilute solutions of iodine with potassium iodide.

From these chemical observations, then, it seems likely that the organic material in heated soil extracts consists mostly of carbohydrate-like substances, probably derived from the cellulose remains of previous plant growth upon the soil. Whatever may be the

source of this material it seems to show properties of sugars and also of organic acids. The cellulose or humus substance seems to be changed by the heat into a series of decomposition products having the groups characteristic of sugars and also of organic acids. Precipitation with normal lead acetate and then with the corresponding basic salt produces a partial separation of these substances, as also does the addition of acetone. The relation of this organic material to the constituents of caramelized sugar would make an interesting study.

CROP EXPERIMENTS

Many experiments with the fungus *Pyronema* had shown that the stronger the extracts of heated soils the better media they were for this fungus. Other authors had reported that the green plants did not take kindly to soils which had been heated to a high temperature. From the above observations it occurred to us that the harmful effects from the heating of the soil in which certain plants are grown might not be due to the fact that the materials rendered available by heating are especially harmful, but that they are formed in such large quantities that the green plant is unable to use them. This view is strengthened by the experience of Livingston¹⁵ and others that growth is accelerated in weak solutions and retarded in concentrated ones. On the other hand, the lower plants such as yeast and fungi are able to grow in solutions that would very rapidly plasmolyze the cells of the higher plants.¹⁶ Evidently, then, the osmotic pressure of their cells must be in equilibrium with that of their medium. In order to test this conception as applied to our problem it was decided to try the effect of the growth of a given plant on the same kind of soil heated to different temperatures. In looking about for a plant to be used in these preliminary experiments, the common oat (*Avena sativa*) was selected for the reason that it had been previously observed that the burning over of the surface of the soil seemed to accelerate the growth of this particular crop.

¹⁵ Livingston: The rôle of diffusion and osmotic pressure in plants, pp. 124-144. 1903.

¹⁶ This matter is discussed in Jost's (Gibson translation) *Plant Physiology*, p. 179. 1907.

Experiment I. Ten pots of common unfertilized New York soil were prepared; two of these were kept as controls and two heated to a temperature of 90° C. for two hours, two to a temperature of 120° for the same length of time, two to 150° and two to 170° C. From one series of pots, extracts were taken by percolating equal amounts of distilled water through each pot. Approximately the same amount came through in each case. One hundred c.c. were taken from each pot to be used for analytical purposes. As has been previously noted, the extracts of heated soils are of a brown color. In this series of experiments it was observed that the color varied directly with the temperature; the control being colorless, the extract from the soil heated to 90° being slightly colored, the color becoming deeper in each case as the temperature increased. The results of the chemical analyses of these extracts are given in Table 1 (page 419).

The other series of pots of soil which had been similarly heated, but not extracted, were watered and planted with oats, the same number of seeds having been placed in each pot. After about two weeks the following results were obtained: the control and pot of soil heated to 90° showed practically the same growth; that heated to 120° showed considerable acceleration; and beyond this the growth was retarded, the retardation in the pot heated to 150° and that heated to 170° being nearly equal. The accompanying photograph (Plate VII) shows the control, the pot heated to 120° C. and that heated to 170° , the contrast being greatest at these points.

Experiment II. In order to check the results obtained in one of the experiments just described, a second series of pots of ordinary New York soil were set up and heated in the manner already stated. In this case the series consisted of an unheated control, and three others heated to 90° , 150° and 180° C. respectively. Oats were planted as usual and their condition noted, after germination and about two weeks' growth had been made. In this series the soil heated to 120° did not show the slight gain over the unheated control that was noted in the earlier experiments, but the higher temperatures produced the same retarding effects already found. In this and previous experiments the soils heated to 120° and above



SEAEVER AND CLARK: GROWTH IN HEATED SOILS.

Left to right: Oats grown in unheated soil, in soil heated at 120° C. and in soil heated at 170° C.

were very soon covered with a luxuriant web of mycelium of *Pyronema* which produced its salmon-colored fruiting bodies and then disappeared. This is another illustration of the fact that conditions of media entirely unsuited for green plants may be highly favorable to fungi. One should be careful not to speak of "toxic" or "poisonous effects" unless the organism is mentioned to which such reference is made.

The cultural experiments just outlined show that heating soils to temperatures above 120° seems to produce effects unfavorable to the growth of oats, while heating to less than 120°, depending, of course, upon the crop and soil used, seems to be slightly beneficial or, at any rate, not definitely harmful. The harmful effects on green plants and the beneficial effects on fungi seem to increase, hand in hand, with the intensity of the heat. This may indicate that both effects are due to the relative *amounts* of substances in solution and not to their so-called harmful or beneficial nature. This parallelism is so complete that it does not seem likely that the effects of heating soils are mainly due to the destruction of *living organisms*—the protozoa mentioned by Russell and Hutchinson or the spores of harmful fungi according to Bolley. In other cultural studies we made extracts of similar series of heated soils for chemical analysis and for culture solutions in lupin experiments now to be outlined.

THE EFFECTS OF SOIL EXTRACTS UPON LUPIN SEEDLINGS

After satisfying ourselves that strongly heated soils were distinctly harmful to the growth of oats upon them, we then used extracts from series of soils heated to different temperatures for equal periods of time as culture solutions for lupin seedlings. These seedlings had been started in germinators filled with sphagnum moss and the radicle allowed to reach a length of 3–5 cm. The seedlings were placed in Jena beakers containing 200–300 c.c. of the extracts to be studied. They were placed on glass rods in lots of four in each extract after the manner described by True and Gies.¹⁷ All measurements are given in millimeters of growth in the designated time and represent the average for *four* seedlings (Table 3).

¹⁷ True and Gies: On the physiological action of some of the heavy metals in mixed solutions. Bull. Torrey Botan. Club, 30, 390. 1903.

TABLE 3. EXPERIMENT I. STARTED MAY 5, 1911
Growth, in Millimeters, of Lupin Seedlings in Soil Extracts

Nature of Extract	May 6	May 8	May 9	May 11
Unheated soil.....	15	43	15	28
Heated to 90°.....	15	48	12	24
Heated to 120°.....	10	24	8	11
Heated to 170°.....	7	17	3	4

Note. For analytic data pertaining to these extracts see page 419 (Table 2).

The appearance and growth of the lupins in the unheated soil extract and the 90° soil extract were apparently normal in every way. However, in the extracts of the 120° and 170° soils the opposite was true. The white roots very rapidly became brownish in color and the formation of secondary roots was almost entirely prevented. Such lupins looked like similar ones acted upon by toxic though minute amounts of copper. The results of another typical experiment upon the effects of the soil extracts are given below.

TABLE 4. EXPERIMENT II. STARTED APRIL 7, 1911
Growth, in Millimeters, of Lupin Seedlings in Soil Extracts

Nature of Extract	April 8	April 10	April 11	April 13
Unheated soil.....	7	24	16	26
Heated to 90°.....	5	22	15	26
Heated to 120°.....	2	16	12	20
Heated to 170°.....	4	12	8	12

Note. Whenever the extracts of heated soils were to be preserved for analysis or seedling studies, it was found necessary to seal them in glass-stoppered bottles while boiling hot.

A glance at the results of our seedling experiments (Tables 3 and 4) shows that, with slight exceptions due to experimental errors, the soils heated to temperatures in the vicinity of 120° to 170° C. contain substances soluble in water which exert a strong inhibitory effect upon lupin seedlings grown in such solutions. The results of our oat culture work are in harmony with the idea, which Lyon also held, that strong heating produces soluble material harmful to green plants. The fact that extracts used for the seedling studies, and the extracts from the oat culture soils, were analyzed and found to have quantitative relations depending on the intensity of the heat, seems to indicate that the causes are to be found in *chemical* changes rather than changes in the soil flora, and so on.

SUMMARY OF CONCLUSIONS

1. The color of the extracts of heated soils varies with the temperatures to which the soils have been subjected; the extract of unheated soil being colorless, that of soil heated at 90° C. being slightly yellowish, that of soil heated at 120° C. being more deeply colored.

2. The amount of soluble matter in the soil also varies with the temperature to which the soil has been exposed and to this the color of the extracts serves as an index; the deeper the color the more soluble matter the extracts contain.

3. The length of time during which the soil has been exposed seems to be of little moment; soil heated at 120° C. for ten hours containing little more soluble matter than the same soil heated at the same temperature for only two hours.

4. Growth of the *green plants* used by us is slightly accelerated in soils heated at low temperatures (90 – 120° C.) but above this point growth is retarded, the retardation increasing with the temperature to which the soils have been exposed.

5. Retardation seems not to be due to the toxic effects of the substances rendered soluble, but to the fact that they are present in such large quantities that the plant is unable to absorb them.

6. The influence of heated soils on the growth of *fungi* is the opposite of that on the growth of green plants, the growth becoming more luxuriant as the temperature is increased. This can be explained by the fact that fungi, unlike most green plants, have the power of adapting themselves to nutrient media of a comparatively high degree of concentration.

7. The beneficial or harmful results, therefore, of the heating of a soil for the growth of plants, depends upon the temperature to which the soil has been heated as well as upon the nature of the soil and the plant.

8. The preference of certain plants for burned-over areas or for peaty soils may be explained by the acidity of such situations, a supposition we are testing by blueberry culture experiments now under way.

Finally, we wish to express our thanks to Professor William J. Gies for his helpful suggestions during the course of our investigations.

THE INFLUENCE OF PHYTIN ON THE GROWTH OF LUPIN SEEDLINGS

A. R. ROSE

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York)

I. INTRODUCTION

Inosite, a cyclic compound, is a very important constituent of all seeds, where it is chemically combined as phytin with phosphate of the alkaline earths. Because this compound (phytin) is stored in seeds and contains more than half of the phosphorus that is available at the beginning of plant growth, we may justly assume that its function in the physiology of plants is highly significant; though as to just what rôle in phyto-mechanism, phytin may play, we have as yet no knowledge. The presence in the seed of a phytase would suggest the correctness of Postenak's inference that phytin is a reserve material designed to supply phosphorus for the process of germination and subsequent growth.

It is now a matter of common knowledge that growing plants require phosphate. In agricultural practice, phosphate is supplied to the soil incidentally, by plowing under many kinds of organic refuse; or intentionally, by adding inorganic phosphates from mineral beds, or the organic fertilizer, guano. Nagaoka¹ investigated the relative values of organic phosphates from animal and from plant sources, and found that the animal products gave better results than the vegetable. His associates, Aso and Yoshida,² later studied the comparative fertilizing values of different vegetable substances containing phosphorus, and demonstrated that phosphoric acid of inosite-hexaphosphate (phytin) is more available for plants than protein-phosphate (nucleoprotein), but less available than fat-phosphate (lecithan). The results of their experiments

¹ Nagaoka: Bull. Coll. Agr., Tokyo, vi, no. 3.

² Aso and Yoshida: Jour. Coll. Agr., Tokyo, i, 1909, pp. 153-161.

are very striking, as is shown below by the averages compiled from their tables.

Phosphorus Container,	Length of Plant. Cm.	Dry Matter. Grams.	Weight of Grain. Grams.
Lecithan	20.4	11.7	4.5
Phytin	11.1	2.5	0.5
Nucleoprotein	9.3	2.3	0.5
Na ₂ HPO ₄	15.9	6.0	1.8
FePO ₄	9.6	3.5	0.8
AlPO ₄	10.2	3.0	0.5
Ca ₃ (PO ₄) ₂	21.0	9.9	3.2
No phosphate	7.8	1.4	0.0

The organic and inorganic phosphates were each added in such amounts as to give the equivalent of 0.395 gram of P₂O₅ per pot of two and a half kilos of soil.

If phytins are normally important constituents of seeds, one might assume *a priori* that these salts, if added to the substrate, would at some stage of the life cycle show an influence on plant growth. Aso and Yoshida have shown from final results that the use of phytin as a fertilizer is not particularly advantageous when compared with the employment of other forms of phosphorus. It remained to be seen whether or not the addition of phytin to the nutrient medium at the beginning of the life of a plant in any instance would cause stimulating effects. Presumably the seed carries sufficient phosphorus for the completion of normal germination, but the presence of an excess over the amount required might reveal some interesting facts. This possibility was tested in the experiments described below.

II. EXPERIMENTAL

General method. Lupin seeds were used in the experiments. They were soaked in water over night. Seeds of practically the same size and plumpness were then selected and planted in wet moss. After three or four days, the seedlings were taken from the moss, one at a time, the coat of each removed, and the sprout rinsed in distilled water. The primary root, now readily discernible, was carefully measured on a millimeter scale.

The seedlings were at once fastened on glass rods drawn out at one end to form a sharp-pointed L and suspended in perforated cork covers over 800 c.c. Jena beakers, each containing 400 c.c. of

solution. The rods were so adjusted in the perforations that the roots were immersed in the solution, but the cotyledons were not in contact with it. Four seedlings were suspended in each beaker. The roots at the beginning differed in length, as much as fifteen millimeters in some cases. An attempt to minimize the effects of such variations was made by placing two shorter roots with two longer ones in the same beaker, or else by choosing what seemed to be the average; four long roots or four short roots were never suspended together. At intervals of one to three days, all the seedling roots were measured.

Variations in growth under "control" conditions. The experiments were carried on before a large window, in a separate room, under as favorable conditions for growth as could reasonably be expected. Irregularities in growth were noticed from the beginning, due to unknown factors. Ordinary distilled water was used in making up the solutions and may account for some of the differences. The chemicals employed were pure, and in each case taken from a dilute stock solution, with the exception of the calcium sulphate and calcium phosphate, which were obtained from large bottles of saturated solutions having excesses of the salts on the bottoms. The calcium sulphate supply was renewed by adding distilled water to the residue in the bottle after each planting, and shaken several times before the day for starting the next set, the required amount being then carefully decanted so as to prevent admixture with the sediment. One bottle of the calcium phosphate solution sufficed for the whole experiment. Characteristic irregularities are indicated in the following tables, the first of which shows the rate of growth of seedlings suspended in a one-half saturated calcium sulphate solution; the second in a 0.06 per cent. calcium nitrate solution.

TABLE I.
"Normal" Growth in 0.12 per cent. $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ Solution

Date of Planting, 1911	Rate of Growth per Plant in Millimeters			
	1st Day	2d Day	6th Day	Average per Day for First Six Days
March 19.....	12	28	6	20.6
April 1.....	8	12	9	8.9
April 24.....	15	16	9	16.5
May 5.....	16	16	8	16.0

TABLE 2.

"Normal" Growth in 0.06 per cent. $\text{Ca}(\text{NO}_3)_2$ Solution

Date of Planting, 1911	Rate of Growth per Plant in Millimeters			
	1st Day	4th Day	6th Day	Average per Day for First Six Days
April 1.....	22	20	11	17.8
April 24.....	14	17	11	12.7
May 5.....	15	8	8	12.3

The individual plants in the several beakers showed no such variation among themselves, except occasionally; when one or two lagged behind the others, they were discarded as defective samples. This shows that factors besides the solutions used play an important part and that it is therefore desirable to have all comparisons of the effects on root growth made on plants grown in the same period of time. If there must be comparisons between plants grown at different times, there should be in each group control tests with solutions of the same chemicals and of the same dilution. In any one period, the growths are quite comparable, as is shown by the data for the following eight tests.

TABLE 3.

Data Showing Comparatively Uniform Rates of Growth in Various Nutrient Media During Given Periods

Date of Planting, 1911	Nutrient Solution	Average Growth per Plant in Millimeters, Days		
		1-4	5-6	7
Mar. 12.....	K_2HPO_4 —0.05 per cent.; KNO_3 —0.01 per cent.; MgSO_4 —0.05 per cent.; CaSO_4 —0.24 per cent.	13	10	17
		13	10	14
Mar. 12.....	Same as above, plus calcium phytate—0.006 per cent.	15	13	12
		14	14	13
		1	2-3	4-5
Mar. 19.....	CaSO_4 —0.13 per cent.; K_2HPO_4 —0.05 per cent.; KNO_3 —0.10 per cent.; MgSO_4 —0.05 per cent.; Ca-phytate—0.04 per cent.	16	24	12
Mar. 19.....	CaSO_4 —0.13 per cent.; K_2HPO_4 —0.05 per cent.; KNO_3 —0.10 per cent.; MgSO_4 —0.05 per cent.; Ca-phytate—0.01 per cent.	17	24	12
Mar. 19.....	CaSO_4 —0.12 per cent.; Ca-phytate—0.005 per cent.	15	24	17
		16	26	17

The particular methods in the studies of the effects of phytin. In distilled water, growth of the seedlings was very slow and ceased after three days. In very dilute solutions of magnesium sulphate, potassium phosphate, or potassium nitrate, results were almost equally negative; but when calcium sulphate or calcium nitrate was used, growth was pronounced. One or the other of these last two substances was therefore used as a sort of "basal ration," and the phytate whose influence was to be studied was added to a solution containing it. Sometimes magnesium sulphate, potassium phosphate and potassium nitrate, substances so commonly used in making artificial soils for experimental purposes, were added, to determine the effect of their association with the phytate.

Many seedlings were planted, some in duplicate solutions, others in media containing various combinations of the compounds mentioned above, many of them without any phytates. From voluminous data a selection has been made of the results which seem to be of the most importance in this connection. The figures which are tabulated below show, in each case, the average growth per plant per day in millimeters, for the period designated, usually from one to three days.

To facilitate comparison between plants grown in different media, there is given in the last column the average growth per plant per day during the first six days, as this covers in all cases the period of most rapid growth. A slight discrepancy between the data in this column and in the others occasionally results from the fact that these averages were made from the recorded data on those plants which flourished to the end of the experiment. Thus, in beaker No. 37, the average per plant per day for the first six days is 12.7 millimeters, whereas the average of the separate day reports would be 12.4 millimeters. These differences are negligible, being very much less than the variations in rate of growth of individual plants in the same beaker. The measurements from period to period are given to show the changes in the rate of growth, because of the depletion of the nutrient medium, or increase of acidity, due to the selective action of the root, or both. (Table 4.)

Discussion of the results. When the seedlings were placed in a very dilute solution of calcium inosite hexaphosphate

TABLE 4.

Data Showing the Effects of Phytin Under Various Nutrient Conditions

Series No. and Date	Beaker No.	Nutrient Solution	Average Daily Growth per Plant in Millimeters, Days				
			1-4	5-6	7	8	First 6 Days
1911 March 12	2	CaSO ₄ .2H ₂ O—0.24 per cent.	11	9	13	13	8.7
	3	CaSO ₄ .2H ₂ O—0.24 per cent.; K ₂ - HPO ₄ —0.05 per cent.	13	10	17	19	10.1
	4	KNO ₃ —0.1 per cent.; MgSO ₄ . 7H ₂ O—0.05 per cent.	13	10	14	11	10.1
	5	CaSO ₄ .2H ₂ O—0.24 per cent.; KNO ₃ —0.1 per cent.	15	13	12	6	11.3
	6	MgSO ₄ .7H ₂ O—0.05 per cent.; Ca- phytate—0.006 per cent.	14	14	13	9	11.3
	7	Same as 5 and 6, with Ca-phy- tate—0.024 per cent.	10	19	10	10	12.1

II			1	2-3	4-5	6-9	First 6 Days
March 19	9	CaSO ₄ .2H ₂ O—0.12 per cent.	12	28	16	6	20.6
	11	CaSO ₄ .2H ₂ O—0.12 per cent.; Ca- phytate—0.006 per cent.	15	24	17	11	19.0
	12	CaSO ₄ .2H ₂ O—0.12 per cent.; Mg- SO ₄ —0.1 per cent.	10	24	17	7	18.5
	13	Same as 12 and Ca-phytate—0.006 per cent.	14	23	18	6	19.3
	14	CaSO ₄ .2H ₂ O—0.12 per cent.; K ₂ - HPO ₄ —0.1 per cent.	12	17	12	6	15.9
	15	Same as 14 and Ca-phytate—0.006 per cent.	17	26	16	8	20.3
	16	CaSO ₄ .2H ₂ O—0.12 per cent.; K ₂ - HPO ₄ —0.05 per cent.; KNO ₃ —0.1 per cent.; MgSO ₄ .7H ₂ O— 0.05 per cent.	14	16	14	7	15.0
	17	CaSO ₄ .2H ₂ O—0.12 per cent.; KNO ₃ —0.1 per cent.; MgSO ₄ . 7H ₂ O—0.05 per cent.; Ca-phy- tate—0.006 per cent.	17	18	15	7	16.6
	18	Same as 17, with Ca-phytate— 0.12 per cent.	16	21	12	7	16.1
	19	Same as 17, with Ca-phytate— 0.36 per cent.	17	24	12	4	18.3
	22	CaSO ₄ .2H ₂ O—0.12 per cent.; Ca- phytate—0.006 per cent.	16	26	17	10	20.8

III			1-2	3-4	5-8	9-12	First 6 Days
March 20	23	CaSO ₄ .2H ₂ O—0.12 per cent.; Ca- phytate—0.03 per cent.	19	12	11	5	14.0
	26	KNO ₃ —0.2 per cent.; Ca-phytate —0.03 per cent.	18	11	7	4	9.9
	28	K ₂ HPO ₄ —0.1 per cent.; Ca-phy- tate—0.03 per cent.	15	16	6	2	13.9

Series No. and Date	Beaker No.	Nutrient Solution	Average Daily Growth per Plant in Millimeters, Days				
			1-2	3-4	5-7	8-10	First 6 Days
1911 April 1	32	Ca(NO ₃) ₂ —0.06 per cent.	17	30	11	8	17.8
	37	Ca-phytate—0.006 per cent.	12	16	10	7	12.7
	38	Ca(NO ₃) ₂ —0.06 per cent.; NH ₄ - NO ₃ —0.015 per cent.; MgSO ₄ · 7H ₂ O—0.015 per cent.; KCl— 0.005 per cent.	13	12	8	4	10.0
	42	Ca(NO ₃) ₂ —0.06 per cent.; NH ₄ - NO ₃ —0.015 per cent.; MgSO ₄ · 7H ₂ O—0.015 per cent.; KCl— 0.005 per cent.; Ca-phytate— 0.006 per cent.	13	17	8	4	10.8
	45	Same as 42, with Ca-phytate— 0.01 per cent.	10	15	5	4	10.0
	46	CaSO ₄ ·2H ₂ O—0.12 per cent.	8	12	12	9	8.9
	47	CaSO ₄ ·2H ₂ O—0.12 per cent.; Ca- phytate—0.006 per cent.	9	19	12	8	10.4

V			1-4	5-6	7	8-11	First 6 Days
April 24	50	Ca(NO ₃) ₂ —0.06 per cent.	14	17	11	6	12.7
	51	Ca(NO ₃) ₂ —0.12 per cent.	13	13	14	7	14.1
	53	CaSO ₄ ·2H ₂ O—0.12 per cent.	15	16	18	9	16.5
	55	CaHPO ₄ —0.005 per cent.	13	15	12	6	13.6
	56	CaHPO ₄ —0.01 per cent.	12	9	12	4	11.6
	57	Ca-phytate—0.006 per cent.	15	13	15	4	12.5
	58	Ca-phytate—0.01 per cent.	15	17	15	4	12.5

VI			1-3	4	5-6	7-10	First 6 Days
April 24	59	Ca(NO ₃) ₂ —0.06 per cent.; Ca- HPO ₄ —0.005 per cent.	15	11	15	8	14.7
	60	Ca(NO ₃) ₂ —0.06 per cent.; Ca- phytate—0.006 per cent.	16	10	5?	10	12.8
	61	Ca(NO ₃) ₂ —0.12 per cent.; Ca- HPO ₄ —0.005 per cent.	15	12	13	12	14.3
	62	Ca(NO ₃) ₂ —0.12 per cent.; Ca- phytate—0.006 per cent.	14	15	12	10	13.2
	63	Ca(NO ₃) ₂ —0.06 per cent.; Ca- HPO ₄ —0.01 per cent.	14	21	13	11	15.0
	64	Ca(NO ₃) ₂ —0.06 per cent.; Ca- phytate—0.012 per cent.	13	13	12	10	12.0
	65	CaHPO ₄ —0.005 per cent.; Ca- phytate—0.006 per cent.	13	14	15	7	13.0
	66	CaSO ₄ ·2H ₂ O—0.12 per cent.; Ca- HPO ₄ —0.005 per cent.	15	15	17	7	16.5
	67	CaSO ₄ ·2H ₂ O—0.12 per cent.; Ca- phytate—0.006 per cent.	15	14	13	9	14.0

Series No. and Date	Beaker No.	Nutrient Solution	Average Daily Growth per Plant in Millimeters, Days				
			1-3	4	5-6	7-10	First 6 Days
VII							
1911 April 24	70	Ca(NO ₃) ₂ —0.06 per cent.; Mg- phytate—0.01 per cent.	12	11	7	9	8? (Very irregular) 7.6
	71	Ca(NO ₃) ₂ —0.06 per cent.; Mg- phytate—0.02 per cent.	12	5	8	8	
	72	CaSO ₄ .2H ₂ O—0.12 per cent.; Mg- SO ₄ —0.03 per cent.	11	6	14	10	11.4
	74	Ca-phytate—0.01 per cent.; Mg- phytate—0.01 per cent.	12	16	7	2	10.0
	75	Ca-phytate—0.006 per cent.; Mg- phytate—0.01 per cent.	11	10	4	1	8.5
	76	CaHPO ₄ —0.01 per cent.; Mg- phytate—0.01 per cent.	13	11	4	0	10.2

VIII			1-3	4-5	6-8		First 6 Days
May 5	77	CaSO ₄ .2H ₂ O—0.12 per cent.	16	14	8		16.0
	78	Ca(NO ₃) ₂ —0.01 per cent.	17	13	9		15.3
	79	Ca(NO ₃) ₂ —0.06 per cent.	15	8	8		12.3
	80	Ca(NO ₃) ₂ —0.12 per cent.	19	12	7		14.8
	81	Ca(NO ₃) ₂ —0.24 per cent.	16	12	10		13.2
	82	CaHPO ₄ —0.0012 per cent.	10	9	1		8.0
	83	CaHPO ₄ —0.0025 per cent.	11	9	4		9.0
	84	CaHPO ₄ —0.005 per cent.	17	9	2		11.5
	85	CaHPO ₄ —0.010 per cent.	14	10	3		11.0
	86	Ca-phytate—0.0005 per cent.	17	8	0		13.6
	87	Ca-phytate—0.001 per cent.	17	9	5		12.0
	88	Ca-phytate—0.002 per cent.	11	9	4		9.6
	89	Ca-phytate—0.012 per cent.	15	7	7		10.9

IX			1-3	4-5	6-8		First 6 Days
May 5	90	CaSO ₄ .2H ₂ O—0.12 per cent.; Mg- SO ₄ .2H ₂ O—0.05 per cent.	15	13	7		13.6
	91	CaSO ₄ .2H ₂ O—0.12 per cent.; Mg- phytate—0.01 per cent.	23	19	17		21.0
	92	Ca(NO ₃) ₂ —0.06 per cent.; Ca- HPO ₄ —0.005 per cent.	22	13	13		18.5
	93	Ca(NO ₃) ₂ —0.06 per cent.; Ca- phytate—0.006 per cent.	20	12	10		14.4
	94	CaHPO ₄ —0.005 per cent.	8	5	0		8.0
	95	Ca-phytate—0.006 per cent.; Mg- phytate—0.006 per cent.	18	11	8		13.2
	96	CaSO ₄ .2H ₂ O—0.12 per cent.; Mg- HPO ₄ —0.0006 per cent.	21	16	9		17.7
	97	CaSO ₄ .2H ₂ O—0.12 per cent.; Mg- phytate—0.006 per cent.	22	19	7		17.5

(varying from 0.0006 per cent. to 0.01 per cent.) root growth progressed in a very healthy manner, as is shown by the results in beakers Nos. 37, 57, 58, 87, 88 and 89. These seedlings, compared with those in the beakers numbered 55-56 and 84, to which similar amounts of CaHPO_4 were added, show a similarity of growth, the averages from the six phytate solutions differing only by one half millimeter from the averages of the three containing calcium phosphate. The seedlings in the solutions of calcium sulphate (Nos. 9, 46, 77) and calcium nitrate (Nos. 32-78), which were planted on the same days as the others just referred to, do not show much improvement over the seedlings in the solutions of calcium phosphate and phytate, in spite of their greater concentration of calcium ions. The initial growths in beakers 78, 84, 86, and 87 are identical, but there is more sustained growth in the solutions which are more concentrated.

The addition of calcium phytate to a solution containing another calcium salt, caused no pronounced change in the rate of growth observed when calcium phytate was absent, as will be seen by comparing the data for breakers 9 and 11 of Series II, and 46 and 47 of Series IV, in which calcium sulphate alone, or calcium sulphate plus calcium phytate, were used. Substitution of calcium phosphate for calcium phytate, as shown in Series VI, resulted in an average root increase of from 1.1 mm. to 3.0 mm. under the influence of the phosphate as compared with the phytate, but this difference is not enough to warrant emphasis.

As stated earlier, solutions containing potassium nitrate, potassium phosphate or magnesium sulphate alone will not stimulate or sustain root growth. If this is due to an inhibitory effect on the part of these compounds, the behavior of the seedlings in beakers Nos. 23, 26 and 28 (Series III) indicates that calcium phytate completely overcomes this adverse influence. The same phenomenon seemed to occur in beakers 11-15 (Series II), where 0.12 per cent. of calcium sulphate was present in each, with or without calcium phytate. In No. 9 of this series is shown, for comparison, the effect of calcium sulphate alone.

When added to mixtures of salts, such as are ordinarily used in laboratories to promote growth for experimental purposes, cal-

cium phytate has no marked influence. This is apparent from the results in beakers 3-7 (Series I), 16-18 (Series II) and 38 and 42 (Series IV). In all these cases, there is only a negligible increase in growth associated with the addition of the phytate. The growth of the seedlings in beaker No. 19 was perceptibly improved, however, when as much as 0.036 per cent. of calcium phytate was added to the mixture used in beaker No. 16.

In all these trials calcium phytate was comparable with dibasic calcium phosphate (CaHPO_4). In both salts, the calcium ion seemed to be the agent which stimulated the growth of the root, and the 0.005 per cent. and 0.006 per cent. solutions were sufficient to supply calcium for more than six days' development.

Besides the calcium salt, magnesium inosite hexaphosphate was also studied in this connection. This compound had an effect upon root growth similar to that of magnesium sulphate, potassium nitrate and potassium phosphate. Data pertaining to the growth of seedlings in solutions containing magnesium phytate along with calcium salts, were obtained in Nos. 70-76 (Series VII). These should be compared with Nos. 59-67 (Series VI) in which various combinations of calcium sulphate and calcium nitrate with calcium phosphate and calcium phytate were employed. In Series VII, the average root growth per plant per day, for the first six days, varies from less than 8.5 mm. to 11.4 mm., whereas, in Series VI, grown at the same time, the range is from 12 mm. to 16.5 mm. Here the retarding effect of the magnesium phytate is plainly indicated.

A comparison of the effects of magnesium phytate and magnesium phosphate has been made in beakers Nos. 96 and 97 (Series IX), calcium sulphate being present in both solutions. No difference in rate of growth occurred.

The double salt of calcium and magnesium (a form of phytin very commonly found in plants) was not favorable to root growth. Various strengths of solution were tried, *e. g.*, 0.001 per cent., 0.002 per cent., 0.005 per cent., and 0.01 per cent. There were growths of 6.0 mm., 9.4 mm., 10 mm. and 7.4 mm. respectively, per plant per day for the first six days, with apparent cessation of growth on the eighth day. The copper salt was found to be exceedingly toxic.

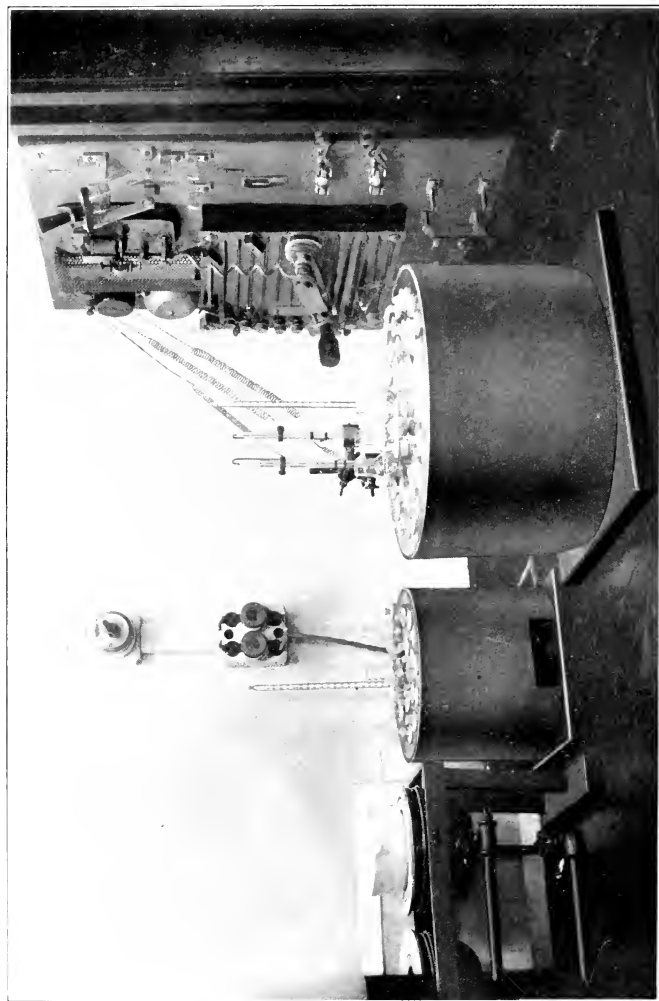
An attempt was also made to determine whether the phytase present in the plant exerts its influence upon phytin in the solution in which the seedling is grown. Twelve seedlings were grown for over twenty days in a beaker containing a large excess of calcium phytate. The solution was then examined for inositol and any increase in the amount of inorganic phosphates, *with negative results*. There was, therefore, no evidence that the phytase acts upon inositol hexaphosphates outside of the organism. A slight increase in acidity was noted, but not determined quantitatively. This phase of the problem will be subjected to further experimentation before any definite conclusion is drawn.

III. GENERAL CONCLUSIONS

The work of Aso and Yoshida³ shows that the soluble organic phosphorus salts, when added to the soil as a fertilizer, are less effective than the inorganic phosphates combined with the same bases. The work described in the present paper shows plainly that the addition of phytin does not cause any stimulation of growth that can be attributed to the phytic acid radical. The behavior of phytates as nutrients in solution is the same as that of inorganic phosphates. Whatever its function may be in the seed during germination and growth, the phytic ion does not exert any marked stimulating influence when added to the substrate in which lupin seedlings are grown, as in these experiments.

The author wishes to acknowledge gratefully his indebtedness to Prof. Gies for this method of research, and for the suggestions to which are due whatever merit this paper may contain.

³ Aso and Yoshida: loc. cit.



WELKER: ELECTRICAL BATHS FOR UREA DETERMINATION.
A picture of the two electrical baths, at the end of an ordinary water bath, in a corner of a hood.

ELECTRICAL BATHS FOR USE WITH BENEDICT'S METHOD FOR THE DETERMINATION OF UREA

WILLIAM H. WELKER

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York)

(With plates 8 and 9)

INTRODUCTION

Benedict's well-known and excellent method for the determination of urea was first published in a preliminary report in 1909.¹ Early in the year of Dr. Benedict's associateship in this laboratory (1909-10), the technic of his method was improved by Dr. Gies's suggested use of a porcelain beaker instead of a glass beaker as the container of the sulfuric acid which Dr. Benedict preferred for the bath.² At Dr. Gies's request, Dr. Benedict taught the method to advanced students in this laboratory prior to the full publication of the details of the method,³ and Miss Olive G. Patterson used it regularly about the same time in a research under Dr. Gies's direction on the partition of urinary nitrogen after external hemorrhage.⁴ Other advanced workers here also had occasion to use the method now and then, before its detailed publication.

During the progress of Miss Patterson's research (during April, May and June, 1910), Dr. Gies found it expedient to devise a number of further improvements in the technic of the method. A lead cap, with perforations large enough to admit the test tubes, was placed over the porcelain bath. This cap obviated the unsatisfactory use of clamps attached to the side of the beaker and inserted into the mouths of the tubes. Jena test tubes, $6 \times \frac{3}{4}$ in. in diameter and bulbous ($1\frac{1}{2}$ in.) an inch below the mouth,⁵ were used

¹ Benedict: Proceedings of the American Society of Biological Chemists, 1909, i, p. 225; also Journal of Biological Chemistry, 1909-10, vii, p. xii.

² Benedict: Journal of Biological Chemistry, 1910, viii, p. 418.

³ Benedict: Ibid., p. 405.

⁴ The results of this research will be published at an early date.

⁵ Bulb tubes, such as those numbered 4,877 in the latest Eimer and Amend general apparatus catalog, were selected.

instead of plain tubes in order to reduce the danger of overflow and loss from excessive ebullition. A large enamelware pan was placed under the bath to retain drippings from tubes taken out of the bath and to catch the acid in case of accidental fracture of the porcelain container.

The latter precaution emphasized an inherent defect in the technic of the method. Although there were no accidents, the necessity for this precaution kept Dr. Gies dissatisfied with the practical operation of the method in spite of the convenience which the above mentioned improvements afforded. It was obvious that an oil bath (an alternative suggested in Benedict's first publication) would be an improvement over the sulfuric acid bath, but the danger of serious accidents from heating an oil bath directly over a bunsen burner was even greater than that from the same treatment of a sulfuric acid bath. The possibility of heating an oil bath satisfactorily on a hot plate over an electric stove appeared to offer a way out of the difficulty. These conclusions did not develop in Dr. Gies's consideration of the matter, however, until his personal experience enforced them and, as the academic year was then about to close, further attention to the matter was postponed.

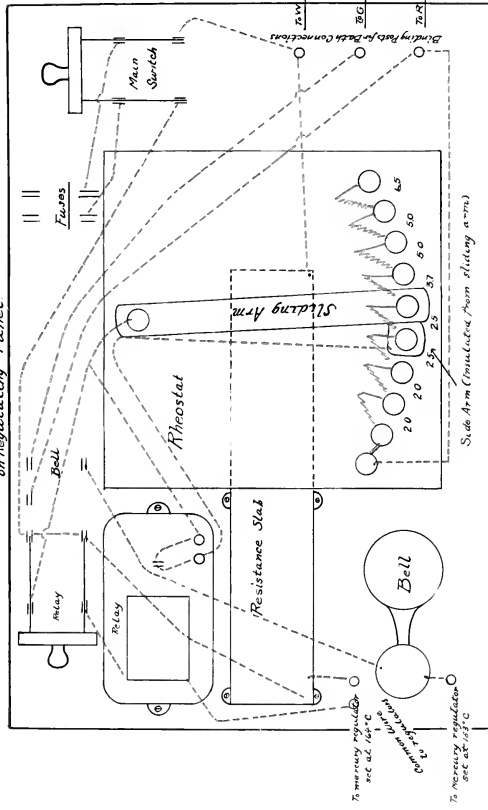
Early in the fall of 1910, at the beginning of my associateship in this laboratory in succession to Dr. Benedict, Dr. Gies requested my coöperation in his plan of improving the technic of Benedict's method, and of constructing apparatus which would facilitate the safe conduct of many determinations by different workers, at the same time and in various stages of the process. Together we then devised the apparatus which is described below. We find that it overcomes all the difficulties and embarrassments to which allusion has just been made. It has steadily been giving perfect satisfaction.

CONSTRUCTION OF THE APPARATUS

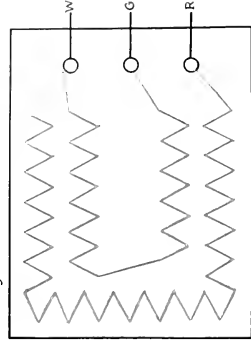
The general character of the apparatus, as placed in working order in one of our laboratory hoods, is shown by the accompanying picture (Plate VIII). The apparatus consists of a *preliminary drying* bath, a *regulating* panel, two *mercury regulators*, and a *constant temperature* bath regulated between 163° and 165° C.

The *preliminary drying* bath consists of a three-heat electrical

*Diagram of Electrical Connections
on Regulating Panel.*



*Diagram
showing arrangement of
Heating Coils and Connections to Bath*



WELKER: ELECTRICAL BATHS FOR UREA DETERMINATION.
Diagram of the electrical connections on the regulating panel.

stove with an 8 inch top. A tin kettle, 4 inches deep and of the same diameter as the stove contains paraffin oil. The tin kettle is surrounded laterally by sheet asbestos $\frac{1}{16}$ of an inch thick and a layer of felt $\frac{1}{4}$ of an inch thick. A metal cover, lined by asbestos board, closes the top of the kettle. Through the metal top and asbestos board are punched $\frac{3}{4}$ inch holes for the tubes.

The *regulating panel* consists of a one inch slate slab, 16 in. \times 23 in., with the following electric apparatus mounted on it: an ordinary electric bell; a relay; a resistance slab; a rheostat; a double throw, double pole switch with two contacts on one side for one arm; two binding posts for line connections; fuses; a double pole switch; three binding posts for the bath connections and three binding posts for the regulator connections. The wiring of the panel and the constant temperature bath is shown in the accompanying diagram (Plate IX).

The *constant temperature* bath consists of a three-heat electrical frying kettle 12 inches in diameter and 4 inches deep. It is about three fourths full of paraffin melting at a low temperature. The heat insulation consists of an asbestos disk under the kettle. Laterally the kettle is coated successively with sheet asbestos $\frac{1}{16}$ of an inch thick, felt wrapping $\frac{1}{4}$ of an inch thick, sheet asbestos $\frac{1}{16}$ of an inch thick, sheet zinc and a felt coat $\frac{1}{4}$ of an inch thick. On the top there is a zinc cover lined by asbestos board. Holes $\frac{3}{4}$ of an inch in diameter have been punched through the cover for the tubes. When not in use these holes are filled with specially turned hard wood stoppers. Bulb test tubes similar to those previously described are employed.

MODE OF OPERATION

The tubes with the urine and reagents⁶ are placed in the preliminary heating bath and the current is turned on. The switch in the bottom of the bath is set so as to allow the maximum amount of current to flow. When the temperature rises to about 100° C., the switch is set to carry the bath to a medium degree of heat. The temperature gradually rises and by the time it reaches 140° C., the tubes are free from moisture. They are then ready for transference to the 164° C. bath.

⁶ Benedict: loc. cit.

The current is turned into the constant temperature bath at the same time that it is directed into the preliminary drying bath. The double throw switch is turned to the bell side, the two heating coils in the bath are thereby connected in parallel and rapid heating of the bath ensues. When the switch is in this position the 154° C. mercury regulator is connected with the bell and the resistance slab. As soon as the bath reaches this temperature, the bell rings. The switch is then thrown to the relay side.

Two mercury regulators are necessary. With only one regulator, the excess of heat in the heating coils sends the bath about 10 degrees above the indicated point, if the switch is thrown when the bath has reached the desired temperature. Therefore two regulators are used, one set about 10 degrees below the desired temperature. If the switch is thrown when 154° C. is attained, the excess of heat in the coils sends the temperature of the bath up to the desired point (164° C.). When the switch is thrown on the relay side, the one coil in the bath is in series with the resistance on the rheostat. The rheostat has attached to its sliding arm, an insulated arm resting on the next lower resistance. This insulated arm is connected through a contact in the relay with the sliding arm. The relay, resistance slab and 164° C. mercury regulator are connected through the relay side of the switch. When the temperature is below 164° , the contact in the relay remains unbroken. When this temperature is reached the current flows through the relay and the connection between the insulated arm with the sliding arm is broken.

It is necessary to ascertain experimentally, where to set the sliding arm on the rheostat so that a little more current than necessary is supplied to the bath when the relay contact is made, and that a little less current than necessary is supplied to the bath when the relay contact is broken. On our apparatus, when in use, the sliding arm of the rheostat rests on point No. 8 and the insulated arm on point No. 7. The resistance slab facilitates the use of "house current" for the bell and relay, and obviates the necessity of employing less reliable battery currents.

The bath maintains great regularity of temperature. In six hour runs it varies less than half a degree. The preliminary desic-

cation and the subsequent dry heating for an hour at 164° C. can easily be accomplished in two hours from the time the current is turned on. The bath for preliminary desiccation holds 16 tubes. The 164° C. bath can take 34 tubes. There is room in the cover for about 12 more openings. The advantages of two baths, for the two different degrees of temperature, are obvious to all who have endeavored to conduct both the desiccation proces and the decomposition operations in one bath at the maximum temperature.

We are indebted to Dr. Eddy for the photographs of the apparatus and for the diagram of the wiring. The electrical regulating panel was constructed for us by the International Instrument Company, Cambridge, Mass.

A RESPONSE TO SOME CRITICISMS OF THE COLLOID-CHEMICAL THEORY OF WATER ABSORPTION BY PROTOPLASM

MARTIN H. FISCHER

(The Joseph Eichberg Laboratory of Physiology in the University of Cincinnati)

Dr. William J. Gies has generously invited me to respond in the pages of this journal to various criticisms and questions he has raised bearing on the general problem of water absorption by protoplasm under various physiological and pathological conditions.¹ The following remarks are in response to this invitation.

If I understand Gies's remarks correctly, he is perfectly agreed to accept as true the statement that the (hydrophilic) colloids of the tissues and the state in which they exist determine in large part the amount of water held by the tissues under various physiological and pathological conditions. If this be true, then Gies and I agree on the main contention of the various papers I have written on this subject in the last six years.² Whether he will accept my belief that the colloids are of such predominant importance in the whole question as to make it appear almost that they alone are involved in the question cannot be gleaned from his remarks, but his adherence to "cell membranes," "osmotic pressure," etc., would indicate that he is less inclined than I to drop the teachings of former decades. Which of these two views is the more nearly correct cannot be determined now. Even though I have chosen the more radical view, I am not ignorant of the fact that no radical in science has ever succeeded in either knowing all the future or in obliterating all the past. Only time can bring the necessary perspective to judge rightly between opposing views or shades of the same view.

¹ William J. Gies: *Biochemical Bulletin*, 1, 124 and 279 (1911 and 1912); F. G. Goodridge and William J. Gies: *Proceedings of the Society for Experimental Biology and Medicine*, 8, 106 (1911).

² See the bibliography at the end of this paper (page 458).

If Gies grants that we agree on the fundamental importance of the colloids and their state in determining the water content of tissues, then a further difference of opinion can arise only in regard to the *mechanism* by which the colloids are enabled to change their water content under different circumstances. But even here I believe that Gies and I must easily find common ground. As the condition in which abnormally great quantities of water are held by the tissues—the edema of the pathologists—is of special interest to medical men and as it is a subject which I have in consequence treated in some detail, Gies has directed most of his criticism toward the mechanism by which I maintain the increased hydration capacity of the tissue colloids in the pathological states that are associated with edema is brought about.

My own conclusions regarding the nature and the cause of edema have been summed up thus:³

A state of edema is induced whenever, in the presence of an adequate supply of water, the affinity of the colloids of the tissues for water is increased above that which we are pleased to call normal. The accumulation of acids within the tissues brought about either through their abnormal production, or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible for this increase in the affinity of the colloids for water, though the possibility of explaining at least some of the increased affinity for water through the production or accumulation of substances which affect the colloids in a way similar to acids or through the conversion of colloids having but little affinity for water into such as have a greater affinity, must also be borne in mind.

To save space, I shall quote Gies⁴ directly and add my comment in as few words as possible.

Gies says: Lactic acid, in Fischer's view, is the responsible acid in the production of edema. One is surprised, therefore, to find that lactic acid does not figure in the summaries of Fischer's *experimental* data. (Page 305.)

³ Martin H. Fischer: *Edema*, p. 99. (New York, 1910.) My earlier papers stating the same conclusion have been and are constantly ignored. See my *Physiology of Alimentation*, p. 268 (New York, 1907); *Journal of the American Medical Association*, 51, 830 (1908); *Kolloidchemische Beihefte*, 1, 93 (1910).

⁴ William J. Gies: *Biochemical Bulletin*, 1, 279 et seq. (1912). All the page references to Gies in the text are to this paper.

Lactic acid is important, though I have never held it to be the only acid involved in the production of edema. It does figure in my summaries, as witness my papers on the swelling of fibrin.⁵ That I did not use it more extensively was determined by the fact that it is not absolutely stabile.

Fischer's experiments involved treatment of colloids and tissues with large and excessive volumes of stagnant acid solutions. He does not show that sufficient organic acid has ever been produced in a given case of edema to cause directly the water accumulation and the swelling observed. (Page 305.)

I used different acids in such widely varying concentrations that the first statement does not seem to me to be entirely true. In answer to the second statement it is sufficient to redirect attention to the analyses of G. Strassburg, A. Ewald and F. Hoppe-Seyler, who found an abnormally high organic acid content in various edema fluids; to H. J. Hamburger's observation that the red and white blood corpuscles swell when carbonic acid is led into arterial blood in sufficient amount to raise it merely to a venous standard; and K. Schorr's finding that a purified protein colloid indicates by an increased swelling (increased viscosity) the difference between the carbonic acid content of conductivity water and of ordinary laboratory water. The protein colloids are probably more sensitive to small changes in acid concentration than some of our finest indicators.

What is the reaction of juice suitably obtained from a typical edematous tissue? Does such liquid contain lactic acid in the free state? (Page 305.)

Such fluid is acid to phenolphthalein always, and I have seen it affect indicators that turn as high as $10^{-5}\text{C}\cdot\text{H}$. Yet this means but little. The protein colloids of the body (which are chiefly responsible for the water held by the tissues) combine chemically with acids added to them. Such acid proteins have an enormously increased hydration capacity and yet indicators hardly serve to show that any acid has been added. So far as the question of the

⁵ Martin H. Fischer and Gertrude Moore: *American Journal of Physiology*, 20, 332 (1907); Martin H. Fischer: *Pflüger's Archiv*, 125, 102 (1908).

causation of edema is concerned, it is not necessary that the acid be "free"; in fact, so far as we know now, it is the "combined" acid that leads to the increased hydration. To satisfy the laws of equilibrium only a theoretical amount of "free" acid need be present.

Does a fluid of this kind have the power of exciting edema in a normal tissue; or of causing such colloids as fibrin, when immersed in it, to swell; or of absorbing more water than that attracted by an equal volume of similar juice from normal tissue of the same kind? (Page 305.)

Heinicke, I think, first showed that the injection of blood serum from a rabbit, rendered nephritic and edematous by injection of uranium nitrate, into a second rabbit made this edematous. But I do not feel that too much weight should be laid upon this finding.

All the water in normal or abnormal blood and lymph is held as hydration water, in combination with the protein colloids. Whether fibrin or any other colloid can take water away from them depends upon whether it has a greater affinity for the water than the colloids of the blood or lymph.

Does the minimal *proportion* of lactic acid which is capable of inducing edema in a living tissue destroy the life of any of the cells or definitely impair normal cellular activity? (Page 306.)

It does not destroy life. Whether it impairs normal "cellular activity" is a matter of definition. A rabbit can be made to hold in water an extra ten or twenty per cent. of its body weight after having been injected with acid, and yet the uninitiated laboratory worker may be unable to recognize any change at all in the animal.

Does such a proportion of lactic acid remove from its setting in protoplasm any basic factor of importance in the essential intracellular coördinations? (Page 306.)

If I understand this remark correctly, I would say that it depends upon the criterion chosen. A little swelling of the liver does not seem to be serious. The same degree of swelling blinds an eye.

In Fischer's view the water of edema diffuses from the capillaries to the tissue spaces and thence into the cells in response to the attraction exerted for it there by intracellular colloids under the

influence of abnormal proportions of acid. Does the production of lactic acid in an edematous tissue keep pace with the inflow and accumulation of water? Does the water which accumulates in a case of edema leave behind it all its former associates in lymph, such as phosphate and bicarbonate, when it enters the edematous field? Does edema result from the action of sodium di-hydrogen (acid) phosphate, or of any other acid salt produced by combinations of intracellular alkalin compounds with any lactic acid that may become available? Does such acid induce edema, or does it combine with or affect protein in any way, *in the presence of an excess of alkalin material?* (Page 306.)

The tissue "spaces" are not empty but filled with water-saturated colloidal material (lymph). Water in the body and substances dissolved in the water travel independently of each other and, at times, in opposite directions. Acid salts act like acids. In the presence of an excess of an alkaline material the acid or the acid salts are converted into neutral salts and then the body colloids under consideration are no longer swelling in the presence of an acid. If the alkaline material is really present "in excess," then alkali-protein will be formed and this has a greater hydration capacity than neutral protein. For a detailed discussion of absorption and secretion, including the question of the formation of lymph, see my paper on this subject.⁶

Fischer demonstrated the striking power of small proportions of electrolytes as agents which counteract the swelling influence of acids, even when the latter are present in large excesses. He does not explain these effects although he emphasizes their importance and their utility. He shows that tartrate and phosphate anions are particularly active in this respect, but he does not appear to consider the probable tendency of phosphate in the tissues to interfere with the swelling influence of such quantities of lactic acid as may be presumed to occur there under pathological conditions. He fails to indicate how much or how little the lactate arising from neutralizations in such cases antagonizes the bloating influences of the lactic acid which, let us assume, keeps on developing in an edematous tissue. (Page 306.)

I have never entered into a detailed discussion of the theoretical physical chemistry of the colloidal state, for Wolfgang Ostwald,

⁶ Martin H. Fischer: *Kolloidchemische Beihefte*, 2, 304 (1911).

Wolfgang Pauli and others can do this better. An edema does not develop as long as the phosphates are capable of counteracting the effect of the acids in the tissues or the lactic acid is changed to a lactate. Edema comes on when this neutralization mechanism has been exhausted. In his intense contemplation of the individual cell Gies often overlooks the complex organism as a whole. When we introduce acid into the body we reduce the total alkali content and therefore the alkali content of every cell and tissue; although for a number of reasons, not necessarily all are altered to the same degree. The blood and lymph are not perpetual fountains of water, alkali and salts, but are readily exhaustible.

Fischer shows that *alternate* treatment of colloids with acids and salins reduces the hydrophilic tendency of the colloids in the presence of acid, but he does not discuss this observation in its relation to similar conditions in cells and tissues.

The diffusion tendencies and effects in edematous tissues are ignored. Does lactic acid accumulate in an edematous tissue as lactic acid or in the form of lactates, or does it pass out of the tissue as acid or as lactates? What is the minimal concentration of lactic acid which is able, in solutions containing the physiological salins in their ordinary proportions, to bring about swelling of any of the colloids or colloidal masses which Fischer used in his experiments? Does this proportion of lactic acid ever occur in edematous tissue? Do *acid* salts favor or retard the tendency of lactic acid to increase the affinity of colloids for water? (Page 306.)

Sins of omission. Some of these points are discussed in papers that have appeared since my book on edema.

Fischer did not consider the probable or possible bearing of definite chemical relationships between the colloids and the acid in the case. Are *definite* unions or relationships between tissue colloids and lactic acid essential to the edematous manifestation of colloidal hydrophilia? If so, do the tissue colloids and lactic acid form a hydrophilic partnership in the presence of such substances as di-sodium hydrogen phosphate and sodium bicarbonate? (Page 307.)

A discussion of this involves us in a discussion of the theory of the colloidal state. Part of the phenomena here are purely physical, as the adsorption phenomena. Others are chemical, as in

the formation of new colloidal compounds when tissue colloids are treated with acids, bases and salts. Even the union between a colloid and its solvent may be chemical. We do not yet know what is the essential nature of hydration, or to express it more generally, of solvation in solution.

Fischer refers to CO_2 as one of the factors in the production of edema but does not show why the large quantities of CO_2 which are normally produced in tissues fail to induce water accumulation in them. (Page 307.)

The carbonic acid concentration in our body cells and tissues is fairly constant. The colloids take up all the water they can hold under these conditions (normal water content). If we permit the carbonic acid content to run up, as through suffocation, atropin or an anesthetic, the animal *does* become edematous *provided we give him water*. If we reduce its carbonic acid content, as by accelerating its heart beat and respiration, by giving caffeine, nicotine or small doses of alcohol, then we reduce the amount of water that its body colloids can hold and so we get "free" water that leaves the body as an increased urinary flow, increased sweat, etc. See my remarks on secretion in my volume on edema and the various papers I have written since.

Does the intracellular CO_2 have any effect directly or indirectly on the power of *lactic acid* to cause edema? (Page 307.)

The two acids act additively. But in the body the production of a stronger acid like lactic will drive off the weaker carbonic acid (acapnia). The ultimate effect on swelling is the algebraic sum of the two in which the nature of the individual acids concerned plays an important part. See the available studies on the effect of different acids on the absorption of water by protein colloids.

The experimental procedure in Fischer's work is not always the best that might have been adopted. Fischer's experiments were performed with *dry* masses, and with solid parts of organisms. How his theory could be *experimentally* verified with blood serum or tissue juice (dissolved colloids) in the absence of "membranes" or "partitions" is not suggested. How do *dissolved* colloids, as compared with solid colloids, behave in the presence of lactic acid, and phosphates, and

bicarbonates? Do the interstitial *dissolved* colloids have any influence in restraining the development of edema at any stage of the process? (Page 307.)

Dissolved colloids behave exactly as do solid ones. Wolfgang Pauli and his co-workers Hans Handovsky, Karl Schorr and Richard Wagner worked this out to perfection so that it would have been superfluous for me to do it also. What I think of the various "membranes" that were born with Pfeffer's osmotic conceptions and which change their nature with every author and every newly gained experimental fact is evident in all my writings.

Goodridge and I find, when moist shreds of fibrin are severally suspended in gelatin solution, peptone solution, fresh egg white, blood, milk, and meat juice, that hydrochloric acid solution (0.2 per cent. to 10 per cent.) may be added to the mixture in each case *in any proportion* without inducing visible effects on the fibrin shreds, *unless sufficient acid is added to provide an excess in the FREE state*. Very large quantities of acid may be added to such mixtures without inducing appreciable bloating effect.⁷ If the colloids in the artificial solutions and protoplasmic liquids enumerated above are combined with any proportion of the acid up to exactly their *maximum* affinity for it (hydrochloric acid), so that the liquids while strongly acid to litmus respond negatively to tests for *free* acid, then moist fibrin shreds can remain in such fluids indefinitely without swelling to any perceptible degree. Warm concentrated gelatin solutions may be put into these conditions of free and combined acidity. After such solutions have been permitted to gelatinize, moist fibrin shreds which have been imbedded in the resultant jellies swell perceptibly, provided the gelatinized mass contains *free* acid, *but the shreds do not appear to absorb water from the medium if its contained acid is only in COMBINED form*. It is obvious that such facts have an important bearing on any theory of acid causation of edema.

A few days ago⁸ I extended these observations to enucleated eyes

⁷ Goodridge and Gies: Proceedings of the Society for Experimental Biology and Medicine, 1911, viii, p. 107.

⁸ "A few days" before the meeting of the Biochemical Association at which this subject was discussed. The results of subsequent experiments will be reported elsewhere. The writer (Gies) has lately commented in a very general way on some of these additional results in a preliminary report in the Proceedings of the Biological Section of the American Chemical Society (BIOCHEMICAL BULLETIN, 1911, i, p. 124). The following remark is included in that report: "Ex-

with similar results and have conducted additional experiments with fibrin and other colloids. After their treatment with 0.05 to 0.2 per cent. hydrochloric acid solution to effect their maximum absorption of water, enucleated eyes and fibrin masses were immersed in combined acid solutions (Witte peptone in 0.2 to 2.0 per cent. hydrochloric acid solution) where they promptly lost all the water they had previously absorbed from the free acid solution and soon returned to the original dimensions. (Pages 307-309.)

I have answered these criticisms before and simply repeat here.⁹ "F. G. Goodridge and William J. Gies [Proc. Soc. Exp. Biol. and Med., 8, 106 (1911)], while apparently accepting the teaching that the colloids of the tissues are responsible for the amount of water held by them, have taken exception to my assertion that an abnormal production or accumulation of acid in the tissues of the body plays an important, if not the chief, rôle in the production of edema, in that these increase the power of certain of the tissue colloids to absorb water. While it would not at all surprise me to have it shown that some other or some series of other changes in the body tissues than an abnormal production or accumulation of acid is responsible for the increased hydration of the colloids here, which is the characteristic feature of edema, the experiments of Goodridge and Gies do not do this. These authors base their criticism on the fact that fibrin threads suspended in such colloidal solutions as gelatin, peptone solution, egg white, blood, milk and meat juice, do not swell visibly on the addition of acid to these solutions, until this is added up to the point where it is "free" in the solution. When these authors add acid to the colloidal solutions in which they immerse their fibrin threads they increase the hydration by this means, not of the fibrin threads, *but of the colloidal solution* (they give this the 'edema'), as they would find if they measured its viscosity. Up to a certain point (maximum hydration under the influence of experiments with enucleated eyes (from dogs, rabbits and chickens, in solutions of *combined* acids) . . . failed to yield edematous results, but emphasized the need for experiments on *solutions* of biological colloids, such as serum and lymph. Fischer's theory is based upon the results of experiments on *solid* masses in large excesses of acid solutions. He has not shown that his experimental conditions are closely analogous to the natural ones in edema." (Page 308.)

⁹ Fischer: Nephritis, p. 184. (New York, 1912.)

the acid) the addition of the acid would therefore tend to *prevent* the fibrin from absorbing water. Only if acid got into it and free water were available could we expect the fibrin thread to swell."

The same may be said of eyes placed in colloidal solutions in which all the water is held as hydration water by the colloids in the solution, where, in other words, no water is "free" from the start or where, under the conditions prevailing in the experiment, none is set "free."

All these results suggest that acid would not cause the *gel* proteins in the cells to imbibe water abnormally in the presence of the associated *sol* proteins. (Page 309.)

We still maintain that it would. As is evident from what we have said, equilibrium always tends to be established between the water in the (*gel*) proteins of the cell and the (*sol*) proteins of the circulating and interstitial lymph or blood. When we increase the hydration capacity of the cell colloids, water tends to move over into them from the blood. If the cell can rid itself of the acid in any way, it becomes unable to hold the excess of water and gives it back to the colloids of the blood and lymph. See my remarks on urinary secretion in my volume on edema and my paper on absorption and secretion.

The results also warrant the provisional inference that the circulating *sol* proteins would attract (and by osmosis obtain) acid of intracellular origin from any combinations there existing with either intracellular *gel* proteins or the intracellular *sol* proteins, or both. (Page 309.)

We would write a question mark after the word "osmosis." With the rest we agree. But there is a limit to the amount of this neutralization. The fact that this mechanism has for a number of reasons been worked to the breaking point is what characterizes the condition that we call edema in pathology.

That the circulating *sol* proteins lose to the associated circulating *basic* compounds any acid combined with or adsorbed to the proteins is a justifiable belief. That the salins resulting from such neutralization reduce hydrophilia in the vicinity of their origin and transit before their excretion, is indicated by many observations. "The acid end

products of metabolism, without appreciably changing the actual alkaline reaction, constantly take up alkali from blood and protoplasm. In this manner there is a tendency to disturb the normal protective equilibrium between bases and acids. This tendency is held in check by the kidney, which in the process of urine formation reverses the reaction of neutralization of acid and restores to the blood that alkali which has served as a carrier of acid."¹⁰ (Page 309.)

We agree again, but I repeat that this neutralization mechanism either mechanically or chemically has been impaired in every condition that is characterized by "edema."

Fischer bases his whole conception on the action of *acid as acid*. That acid, *as acid*, is the responsible and aggressive agent in the production of any *natural* edema is something that I cannot see.

A criticism of my ability to express myself clearly. To avoid dispute, I have never said more than that the acid *content* of the cells is raised in edema. Very evidently the acid need not remain there "as acid." It may neutralize basic salts, combine with protein, and in other ways distribute itself between the different phases of the cell.

On the other hand, that acid by reducing basicity or effecting a reaction-discoördination or inducing some other molecular disequilibrium, may be an *inciting* cause, or a *stimulating* influence, or an *indirect* though none the less influential factor, is quite comprehensible. As a *link* in a *chain* of factors, the influence of acid in effecting abnormal hydrophilia is conceivably important. (Page 309.)

As far as I understand this I agree entirely.

Fischer's book has the great merit of sharply stimulating questions. Do *any non-acid products* of intermediary metabolism retard or accelerate the presumed action of lactic acid in edema? Fischer does not discuss this matter. Has it been definitely established, directly or by methods of exclusion, that nothing occurs in an edematous part but the production of organic acid to account for an increased affinity of the colloids for water? (Page 309.)

Not at all. This is a field for research.

Has Fischer duly considered, in this connection, the effect of patho-

¹⁰ Henderson: Journal of Biological Chemistry, 1911, ix, p. 423.

colloidal coördinations in cells, in response to various prevailing influences in incipient edema? (Page 310.)

I do not understand this.

Does it follow, because acids increase colloidal hydrophilia and because lactic acid production is increased by suboxidation in tissues as edema is there inaugurated, that the edema is caused or initiated by the resultant lactic acid? (Page 310.)

A question in logic. This could be answered correctly by either yes or no.

Have the possible influences of hydrolases been duly considered? Is it improbable or impossible that such enzymes are even more important factors in the development of edema than the acid which is produced and to which Fischer attributes the whole drops? (Page 310.)

Ferments have been considered. Whether they are as important as the acid or more so, or whether acids and ferments act together, cannot yet be said, for *quantitative* studies of the subject were lacking when I wrote on edema, and are still lacking. When they have been made—and work has been progressing on this subject for a year past in our laboratory—the answer will be easy.

May not enzymes of this kind—aided perhaps by organic acids or acid salts or both—cause such changes in the normal intracellular colloidal coördinations and in the colloids themselves as to result in increasing the total affinity for water by the parts involved? (Page 310.)

Certainly, and I have said so repeatedly in my various books and papers.

Are the hydrostatic phenomena of certain edemas clarified by Fischer's conception of directive colloidal hydrophilia? (Page 310.)

I think so. Body position influences the circulation, which in turn influences the oxygen supply to a part, etc., as I have previously pointed out.

Does his theory account for the great diversity in composition of edematous fluids? Repeated severe hemorrhage, on a free diet, is followed by the urinary excretion of lactic acid in exceptional quantities but there is no visible anasarca. Why not?

The first is a special question I do not care to discuss at the present time. Repeated hemorrhage on a free diet is followed by edema. An animal may have a great edema before it evidences itself as a "visible anasarca."

What is the explanation of the absence of general edema in diabetic "acidosis"?¹¹ (Page 310.)

That about 40 per cent. of the true diabetics with acidosis show an obvious edema is a generally recognized clinical fact. Rollin T. Woodyatt found in a series of careful studies on diabetics that their body weight increased or diminished a kilo or two in twenty-four hours when, through diet, their acidosis was permitted to run up or down.

Do all observers agree that "the fluid of an edematous tissue is very decidedly in the cells themselves?" (Page 310.)

I did not say *only* in the cells. This phrase was used in combatting the notion that cells have "membranes" of an osmotic or lipid character about them. It was used when I was maintaining that the absorption of water or any dissolved substance by a cell anywhere in nature was not yet explained when we had succeeded merely in getting these substances through such a hypothetical overcoat about the cell. I have everywhere in my writings emphasized more than any other author the importance of all the intercellular substances, whether solid or liquid, and have made them rank as of equal importance with the cells themselves in this whole problem of the absorption and secretion of water and of dissolved substances.

Is there no excess of interstitial water in edema? (Page 311.)

There is, for the interstitial solid or liquid colloids swell, if a source of water is available, just as do the cells that are composed of the same material.

¹¹ If general edema fails to occur in diabetes because of neutralization of the "diabetic acids" or if the acids of diabetic "acidosis" merely neutralize abnormal basicity, in what material respects are these neutralization and inhibitive effects different from those that normally prevail in practically all parts of the living body? Surely, since large proportions of non-electrolytes are practically without inhibitive effects on colloidal attraction for water in the presence of free acid, "diabetic sugar" would not cause the observed difference. (Page 310.)

Are the *fibrillæ* of an edematous connective tissue bloated? (Page 311.)

The connective tissue contains an increased amount of water.

According to Fischer a frog leg immersed in water becomes edematous as a result of postmortem acid formation primarily (autolytic hydrolysis of carbohydrate?) and saline dialysis secondarily (removal of inhibitive factors). But what about the possible effects of *general* autolysis with its consequent protoplasmic discoordinations and preliminary cumulative productions of additional hydrophilic molecules? (Page 311.)

I agree. But in the absence of quantitative studies no one yet knows what relative importance is to be assigned to each of the phenomena.

Fischer does not touch on the well-known effects of the various lymphagogues. This is an interesting and important omission. Twelve years ago Asher and I¹² observed prolonged *postmortem* flow of thoracic lymph from a dog. Others have since obtained similar results. What in Fischer's view is the bearing on edema of such phenomena of lymph flow? (Page 311.)

My ideas on lymph formation cannot be discussed in the space allowed me here. I have already dealt with the problem in my paper on secretion and absorption.

Does Fischer's theory explain "*œdema ex vacuo*"? Are hereditary edemas or neuropathic edemas, natural and experimental, readily explained by this theory? (Page 311.)

Modern pathologists have often wished that the term "*œdema ex vacuo*" had never been coined, for it says nothing. If the colloidal theory of edema is really correct it will explain the "hereditary" and "neuropathic" types even though the mechanism by which these are brought about is not yet entirely analyzable. Neither of these types is as "hereditary" or as "neuropathic" as the older pathologists and clinicians were wont to believe. "Neuropathic" edemas have not yet been produced "experimentally."

¹² Asher and Gies: *Zeitschrift für Biologie*, 1900, xl, p. 207.

The remaining pages (311 to 315) of Gies's criticism quote various passages from my book in which I touch upon the rôle of various enzymes in increasing the hydration capacity of the tissue colloids, and emphasize his view that in these is to be sought a more potent cause for the production of edema than in the acids which I have discussed in particular detail. I am perfectly ready to admit that this may be so, but to repeat what I have already said, in the absence of quantitative studies no one can yet say which of the various factors that may be active are most active.

* * *

Dr. Gies has tried to deal so conscientiously with my work that it will not be regarded as personal criticism if I append to this article a list of my various publications, with their titles, that I have written from time to time on this general problem of water absorption in biological material under physiological and pathological conditions. Many a warm criticism that has been launched against me has no better foundation than that the author has found it inconvenient to read more than one of my publications. The American reader scarcely deserves blame for this, for the various articles I have tried to publish here have been returned by the editors. I have tried to repeat myself as little as possible in my various writings. Perhaps future critics, if they trouble to enter the discussion at all, will, before publication of their views, study the original sources as carefully as Dr. Gies has generously done.

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STUDIES OF ENZYMES AS POSSIBLE FACTORS IN THE DEVELOPMENT OF EDEMA

I. Further comment on Fischer's theory of edema

WILLIAM J. GIES

(Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York)

Professor Fischer's paper in this number of the *BIOCHEMICAL BULLETIN*¹ is a very gratifying addition to the literature on the "collochemical theory of water absorption by protoplasm." It removes much of the doubt that existed when my "questions" were raised.²

Fischer and I are in accord in our views on the following main propositions:

1. The *hydrophilic colloids* of the tissues, and the state in which they exist, determine *in large part* the amount of water held by the tissues under various physiological and pathological conditions (Fischer, page 444).³

2. Fischer states that "*the accumulation of acids within the tissues*, brought about either through their abnormal production or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible (in edema) for the (abnormal) increase in the affinity of the colloids for water" (page 445). We agree that it is *not* "*acid as acid*" which accomplishes such results (page 454).⁴

Fischer concludes that the responsible acid "may neutralize

¹ Fischer: *Biochemical Bulletin*, 1912, i, p. 444.

² Gies: *Ibid.*, 1911, i, p. 305.

³ Page numerals in parenthesis in this paper indicate pages in *this number* of the *Biochemical Bulletin*.

⁴ At one place in his remarks Fischer states (page 448) that "acid salts act like acids. In the presence of an excess of an alkaline material (*e. g.*, in blood and lymph) the acid or the acid salts are converted into neutral salts and *then* the body colloids under consideration are no longer swelling in the presence of an acid."

basic salts, combine with protein, and in other ways distribute itself between the different phases of the cell" (page 454). He accepts with approval, "so far," he says, "as he understands it," and quotes (page 454) my suggestion that "acid, by reducing basicity or effecting a reaction-discoördination or inducing some other molecular disequilibrium, may be an *inciting* cause, or a *stimulating* influence, or an *indirect* though none the less influential factor" in the production of edema; that "as a *link* in a *chain* of factors, the influence of acid in effecting abnormal hydrophilia is conceivably important."

3. *Enzymes* (such as hydrolases) may determine *in large degree* the amount of water held by the tissues under various physiological and pathological conditions (Gies, page 455).⁵ "Whether acids and ferments act together cannot yet be said, for *quantitative* studies of the subject . . . are lacking" (Fischer, page 455).⁶

Our agreement on the three general propositions formulated above emphasizes the following additional facts:

A. Fischer originally⁷ attributed edema *primarily* to an "*accumulation of acids* within the tissues" but gave *merely casual consideration* to several experimental observations which suggest that enzymes are possible factors of influence in this relation.

B. While I have said, in agreement with Fischer, that acid "may be an *inciting* cause, or a *stimulating* influence, or an *indirect* though none the less influential factor" in this connection (page 454), I have also *been urging special attention to enzymes as agents that may possibly equal, if not exceed, acid in importance in the causation of edema*. I have not assumed that the supposed action of enzymes is *dependent* on acidity or on reduced basicity, or on any one type of conditions.

By concluding that "*quantitative studies*" of the relative powers of acids and enzymes will make it easy to "answer" the main

⁵This general statement about the possible influence of enzymes, in the words of Fischer's deduction regarding the rôle of acid, is embodied in the writer's comment on pages 312-315 of the Biochemical Bulletin, 1911, i (No. 2).

⁶A previous paper from this laboratory recorded observations in this connection which are significant. See Berg and Gies: Journal of Biological Chemistry, 1907, ii, pp. 508, 522 and 545. *Alkalies* may be as effective as acids. (See also the succeeding paper by Tracy and Gies, page 467.)

⁷The writer refers to Fischer's book on edema.

question I have raised, and that such studies are desirable (page 455), Fischer accepts the essential point in my friendly criticism of his theory.

The paper following this one⁸ presents additional *experimental* data on the possible influence of enzymes as factors in the production of edema.

Lack of space in this issue of the BULLETIN prevents full discussion of Fischer's answers⁹ to my "questions,"¹⁰ but the following brief statements relate to a few of the subjects which I feel should be considered here.

When I remarked, in my original criticism of Fischer's statement of his theory of edema,¹¹ that "one is surprised to find that lactic acid does not figure in the summaries of Fischer's experimental data" (page 445), I was referring to the summaries *in the book on edema then under review*. I regarded the book on edema as Fischer's best and most complete statement of his theory. I knew of Fischer's earlier results with lactic acid but I thought his failure to use lactic acid data *in his book* was singular, in view of the importance he has been ascribing to lactic acid as the leading direct factor in the production of edema.

Fischer says (page 449): "In his intense contemplation of the individual cell, Gies often overlooks the complex organism as a whole." This remark appears to ignore my emphasis on the *influence of the general circulation and the kidneys* in maintaining "the normal protective equilibrium between bases and acids" in the individual cells and tissues (page 454).

Fischer's remarks on the results and conclusions published by Goodridge and Gies (page 452) miss the point we believed our statements emphasized.¹² I said, *in my review of Fischer's book*, after restating the findings by Goodridge and Gies (page 454): "All these results (with "*combined acid*") suggest that acid would not cause the *gel* proteins in the cells to imbibe water abnormally in the pres-

⁸ Tracy and Gies: *Biochemical Bulletin*, 1912, i, p. 467.

⁹ Fischer: *loc. cit.*

¹⁰ Gies: *loc. cit.*

¹¹ Gies: *loc. cit.*

¹² Fischer's quotation is taken from a brief preliminary report, which was not as detailed as we would have preferred to make it.

ence of the associated *sol* proteins.¹³ The results also warrant the provisional inference that the circulating *sol* proteins would attract (and by osmosis obtain) acid of intracellular origin from any combination there existing with either intracellular *gel* proteins or the intracellular *sol* proteins, or both."¹⁴ In the experiments performed by Goodridge, our protein solutions were analogous to natural circulating liquids containing *sol* proteins (*e. g.*, blood, lymph, intracellular fluid) and the fibrin was comparable to the *gel* proteins in cells and tissues. The greater affinity of the *sol* proteins for water, after treatment with acid, was apparent in all our experiments. For the very reason that the protein liquids which received and combined acid *were rendered edematous by the treatment, and refused to give up water to the immersed gel proteins* (fibrin, eye), we believed circulating lymph and blood *would tend to keep cells from becoming edematous* by removing acid from the cells as fast as it would form. We were suggesting that the circulating and interstitial *sol* proteins, by becoming edematous locally and moving away, would prevent edema in the cells. This was the significance of the following sentence with which Fischer concludes his quotation from our remarks in this connection (page 452): "After their treatment with 0.05 to 0.2 per cent. hydrochloric acid solution to effect their maximum absorption of water, enucleated eyes and fibrin masses were immersed in *combined acid* solutions (Witte peptone in 0.2 to 2.0 per cent. hydrochloric acid solution),¹⁵ *where they promptly lost all the water they had previously absorbed from the free acid solution* and soon returned to the original dimensions."¹⁶ I have also believed that although *accumulation* of acid of intracellular origin might be prevented by lymph and blood, yet intracellular hydrolases

¹³ To this remark Fischer replies that he "still maintains that it would." (See the next footnote.)

¹⁴ To this remark, which is a direct continuation of the preceding one in the original, Fischer responds: "We would write a question mark after the word 'osmosis.' *With the rest we agree.*" (See the preceding footnote.)

¹⁵ These liquids had been rendered edematous. They were similar to circulating blood and lymph in their capacity to combine with acid and attract water.

¹⁶ When Fischer says (page 457) "the connective tissue contains an increased amount of water," he does not answer this question asked of him: "Are the *fibrilla* of an edematous connective tissue bloated"?

(produced cumulatively perhaps, or progressively freed from the restraining influence of anti-hydrolases) would probably remain *in situ* and give increasing intracellular evidence of their power to excite water imbibition.

Experiments begun in the fall of 1910, with the coöperation of Mr. John L. Kantor and which I have carried forward occasionally since that time, indicate that protein-acid ("combined acid") solutions,¹⁷ which, like those used by Goodridge and Gies, do not induce swelling of fibrin or collagen masses, *promptly acquire that power when pepsin is dissolved in them*. I expect to publish the details of these and related experiments in the near future.

In reply to my reference to "neuropathic edemas, natural and experimental," Fischer says that such edemas have not yet been produced "experimentally" (page 457). When I wrote my remarks in this connection I had in mind the following statement by Wells in a discussion of "neuropathic edema":¹⁸ "That nervous control is a possible factor is well shown by many experiments; for example, simple ligation of the femoral vein in animals does not cause edema, but if the sciatic nerve is cut the vasoconstrictors are paralyzed, and edema may follow (Ranvier). In this case the nervous influence is only indirect, through its vasomotor effects. Similarly, stimulation of vasodilator fibers may cause edema." Furthermore, I understood that "angioneurotic edema has affinities with urticaria, the giant form of which is probably the same disease. . . . Quincke regards the condition as a vasomotor neurosis, under which the permeability of the vessels is suddenly increased."¹⁹ That angioneurotic edema and urticaria may be anaphylactic phenomena, involving disturbance of vascular tonus, was also brought to mind by very interesting results of experiments in this laboratory which Dr. Oscar M. Schloss will describe in the near future.

Fischer states that he "does not understand" the following question in my review: "Has Fischer duly considered, in this connection (the production of edema), the effect of pathocolloidal²⁰

¹⁷ These are *edematous* protein-acid solutions.

¹⁸ Wells: Chemical Pathology, 1907, p. 294.

¹⁹ Osler: Principles and Practise of Medicine, 1911, p. 1104.

²⁰ Pathocolloidal is an analogue of collochemical, coined for the occasion. The phrase might have been put more directly as follows: the effect of pathological coördinations of colloids in cells, etc.

coördinations in cells, in response to various prevailing influences in incipient edema?" (page 455).

This question is vaguely general, I admit. I had in mind, especially, changes in the relations between enzymes and anti-enzymes, increased or decreased content of inorganic radicals in the colloids, new combinations or cleavages of colloids and colloid complexes, and other possible colloidal conditions connected with the inauguration of edema by whatever influence might be initially operative.

I shall take occasion to return to some of these subjects and to remaining points in Fischer's rejoinder, when I discuss the results of various series of experiments which are now nearing completion.

STUDIES OF ENZYMES AS POSSIBLE FACTORS IN THE DEVELOPMENT OF EDEMA

2. The influence of proteases on the swelling of fibrin, collagen and elastin particles in alkaline and acid media

GROVER TRACY AND WILLIAM J. GIES

(Laboratory of Biological Chemistry of Columbia University, at the College
of Physicians and Surgeons, New York)

INTRODUCTION

Six years ago Berg and Gies completed a preliminary series of experiments on "the effects of ions on catalysis, with particular reference to peptolysis and tryptolysis."¹ The following remarks appear in their "summary of main conclusions" (page 545):²

In general harmony with the observed digestive disparities, there were marked inequalities of the swelling effects on fibrin in every equivalent series of acid or basic solutions. Bloating influences on fibrin were due primarily to the acid or base, but were *more pronounced in the presence of enzyme*. Elastin did not swell perceptibly in either the acid or basic solutions employed, but *did so in the latter when trypsin was present*.

The parts played by the various ions and molecules in peptolysis, or tryptolysis, or in the *swelling of the proteins*, have not been considered experimentally in any *special* way. Whether the ions or molecules or both affect the proteins, the enzymes, *the water*, or all collectively, in the swelling and hydrolytic processes, will be considered at a more favorable opportunity.

The "more favorable opportunity" for *extended* consideration of the "swelling and hydrolytic processes" has not arisen, although

¹ Berg and Gies: Journal of Biological Chemistry, 1906-7, ii, p. 489.

² References to the experimental observations on which the above conclusions are based appear on pages 508, 509, 522, 523 and 541. Under the head of "swelling effects on fibrin," Berg and Gies wrote (page 523): "We intend to repeat the experiments under various conditions and to discuss the significance of the results after more data have been accumulated."

we have occasionally been at work on various aspects of these subjects. Berg's "comparative study of the digestibility of different proteins in pepsin-acid solutions"³ was the first and most detailed subsequent contribution in this connection. At various times during the last two years, Miss Tula L. Harkey, Professor Wm. H. Welker, Dr. F. G. Goodridge, and Messrs. John L. Kantor, Samuel Gitlow, Edgar G. Miller, Jr., and the junior author have coöperated in extensions of the work. The results will be prepared for publication as soon as it is convenient to do so. We present in this paper the results of some of the experiments which were performed by the junior author during the past winter.

EXPERIMENTAL. GENERAL PROCEDURE

The preliminary observations by Berg and Gies,⁴ on the power of proteases to increase protein hydrophilia in acid and alkaline solutions, have been confirmed by *quantitative* methods in these experiments. The absolute amounts of water absorbed by weighed quantities of comparatively pure protein products, from given acid and alkaline enzyme solutions, were ascertained by accurate measurements of the volumes of the corresponding filtrates obtained *under conditions of experimental uniformity*.⁵ Typical summaries from our records in this connection are appended.

FIRST SERIES OF EXPERIMENTS

With fibrin and pepsin in 0.1 per cent. hydrochloric acid solution

1. **Conditions of the first experiment:** Weighed quantities of fibrin (3 grams) were transferred to six tall narrow glass-stoppered graduated bottles (250 c.c.). The fibrin was treated there, in each case, with 250 c.c. of 0.1 per cent. hydrochloric acid solution. To each mixture, except the control, a definite amount of dilute pepsin⁶ solution was added promptly, and the contents of the bottle were gently shaken at once. The concentration of the pepsin product in the stock solution specially prepared for use in the experiment

³ Berg: *American Journal of Physiology*, 1909, xxiii, p. 420.

⁴ Berg and Gies: *loc. cit.*

⁵ Brod: *Beiträge zur Lehre von der Eiweissverdauung*. Dissertation, Würzburg, 1892.

⁶ An ordinary commercial product was used in each experiment.

was 0.008 per cent. (0.1 gram per 1,200 c.c.). The mixtures were kept at room temperature for two hours before filtration was started. The exact nature of each mixture in the group, and the experimental and calculated data, are summarized in Table 1.

TABLE 1.

No.	Character of the Mixture			Filtrate, C.c.	Amount of Water Absorbed		
	Fibrin, Grams	Hydro- chloric Acid Solution : 0.1 Per Cent., C.c.	Pepsin Solution —0.008 Per Cent., C.c.		Total, C.c.	Excess over the Control	
						Absolute, C.c.	Relation to the Weight of the Fibrin, Per Cent.
1	3	250	None (control)	169	81	—	—
2	3	250	0.15	160	90	9	300
3	3	250	0.30	160	90	9	300
4	3	250	0.45	158	92	11	366
5	3	250	0.60	159 ⁷	91	10	333
6	3	250	0.75	160 ⁷	90	9 ⁸	300

The data of this experiment show that pepsin markedly increased the water-combining power of fibrin under the imposed conditions. The striking power of the pepsin in this experiment is fully appreciated when the active proportion of the enzyme is considered. The concentration of the *commercial pepsin product* in the liquid that exhibited the greatest bloating power was 0.000,014 per cent. The proportion of *pure pepsin* in that liquid must have been very much less.

2. **Conditions of the second experiment:** Same as those of No. 1, except that the concentration of the pepsin product in the enzyme solution was 0.05 per cent. (0.1 gram in 200 c.c.). See Table 2.

3. **Conditions of the third experiment:** Same as those of Nos. 1 and 2, except that the concentration of the pepsin product in the enzyme solution was 0.1 per cent. (0.1 gram in 100 c.c.). See Table 3.

In the second and third experiments the heights of the columns of bloated fibrin indicated that the enzyme *accelerated* the swelling

⁷ Slight digestion.

⁸ The differences in the volumes of the filtrates in all the experiments were less striking than the differences in the heights of the columns of bloated protein materials.

process. The concentration of the enzyme was obviously sufficient, however, to effect marked digestion of some of the material before the fibrin absorbed the maximum proportions of water.

TABLE 2.

No.	Character of the Mixture			Filtrate, C.c.	Amount of Water Absorbed	
	Fibrin, Grams	Hydrochloric Acid Solution : 0.1 Per Cent., C.c.	Pepsin Solution—0.05 Per Cent., C.c.		Total, C.c.	Less than the Control, C.c.
1	3	250	None (control)	168	82	—
2	3	250	0.15	169 ⁹	81	1
3	3	250	0.30	169 ⁹	81	1
4	3	250	0.45	170 ¹⁰	80	2
5	3	250	0.60	172 ¹⁰	78	4
6	3	250	0.75	173 ¹⁰	77	5

TABLE 3.

No.	Character of the Mixture			Filtrate, C.c.	Amount of Water Absorbed.	
	Fibrin, Grams	Hydrochloric Acid Solution : 0.1 Per Cent., C.c.	Pepsin Solution—0.1 Per Cent., C.c.		Total, C.c.	Less than the Control, C.c.
1	3	250	None (control)	170	80	—
2	3	250	0.15	170 ¹¹	80	0
3	3	250	0.30	170 ¹¹	80	0
4	3	250	0.45	174 ¹²	76	4
5	3	250	0.60	176 ¹²	74	6
6	3	250	0.75	178 ¹²	72	8

4. **Conditions of the fourth experiment:** Same as those of Nos. 1, 2 and 3, except that the concentration of the pepsin product in the enzyme solution was 0.0125 per cent. (0.1 gram in 800 c.c.). See Table 4.

The concentration of the enzyme, in some of the mixtures in the fourth experiment, favored an exhibition of increased swelling by the fibrin *before fully compensatory digestion could occur*. The enzymic conditions of the fifth experiment, and those of the first, were more favorable for this occurrence than those of the remaining experiments in this series (I).

⁹ Slight digestion.

¹⁰ Marked digestion.

¹¹ Slight digestion.

¹² Marked digestion.

TABLE 4.

No.	Character of the Mixture			Filtrate, C.c.	Amount of Water Absorbed		
	Fibrin, Grams	Hydrochloric Acid Solution: 0.1 Per Cent., C.c.	Pepsin Solution—0.0125 Per Cent., C.c.		Total, C.c.	Excess over the Control	
						Absolute, C.c.	Relation to the Weight of the Fibrin, Per Cent.
1	3	250	None (control)	170	80	—	—
2	3	250	0.15	170	80	0	0
3	3	250	0.30	168	82	2	66
4	3	250	0.45	167	83	3	100
5	3	250	0.60	169 ¹³	81	1	33
6	3	250	0.75	169 ¹³	81	1	33

5. **Conditions of the fifth experiment:** Same as those of No. 1, except that a *different preparation of fibrin* was used. See Table 5.

TABLE 5.

No.	Character of the Mixture			Filtrate, C.c.	Amount of Water Absorbed		
	Fibrin, Grams	Hydrochloric Acid Solution— 0.1 Per Cent., C.c.	Pepsin Solu- tion—0.008 Per Cent., C.c.		Total, C.c.	Excess over the Control	
						Absolute, C.c.	Relation to the Weight of the Fibrin, Per Cent.
1	3	250	None (con- trol)	164	86	—	—
2	3	250	0.15	161	89	3	100
3	3	250	0.30	159	91	5	166
4	3	250	0.45	159	91	5	166
5	3	250	0.60	160	90	4	133
6	3	250	0.75	161 ¹³	89	3	100

SECOND SERIES OF EXPERIMENTS. WITH ELASTIN

Group A. Trypsin in 2.5 or 5 per cent. ammonium hydroxid solution

6. **Conditions of the sixth experiment:** Same as those of Nos. 1–5 (first series), except that finely divided elastin (3 grams) from *Ligamentum nuchæ* (oxen) was substituted for fibrin, 5 per cent. ammonium hydroxid solution for 0.1 per cent. hydrochloric acid solution, and commercial trypsin for ordinary pepsin. Further, the dry trypsin was added to the dry elastin in each bottle and the

¹³ Slight digestion.

alkalin solution poured upon them. The mixtures were kept at room temperature six hours before filtration was started. See Table 6.

TABLE 6.

No.	Elastin, Grams	Ammonium Hydroxid Solution : 5 Per Cent., C.c.	Trypsin, Mg.	Filtrate, C.c.	Amount of Water Absorbed		
					Total, C.c.	Excess over the Control	
						Absolute, C.c.	Relation to the Weight of the Elastin, Per Cent.
1	3	250	None (control)	226	24	—	—
2	3	250	100	223	27	3	100
3	3	250	200	222	28	4	133
4	3	250	300	220	30	6	200

7. **Conditions of the seventh experiment:** Same as those of No. 6, except in the strength of the alkali (2.5 per cent.), the weight of elastin taken (5 grams), and in the length of time before filtration was started (6½ hr.). See Table 7.

TABLE 7.

No.	Elastin, Grams	Ammonium Hydroxid Solution: 2.5 Per Cent., C.c.	Trypsin, Mg.	Filtrate, C.c.	Amount of Water Absorbed		
					Total, C.c.	Excess over the Control	
						Absolute, C.c.	Relation to the Weight of the Elastin, Per Cent.
1	5	250	None (control)	214	36	—	—
2	5	250	100	212	38	2	40
3	5	250	200	208 ¹⁴	42	6	120
4	5	250	300	210 ¹⁴	40	4	80

Experiment B. Trypsin in 1 per cent. sodium carbonate solution

8. **Conditions of the eighth experiment:** Same as those of No. 6, except that 1 per cent. sodium carbonate solution was used instead of 5 per cent. ammonium hydroxid solution, and the duration of the experiment was nine hours. See Table 8.

The results of the experiments with trypsin and elastin are the same in principle as those with pepsin and fibrin (*first series*), and

¹⁴ Slight digestion.

with pepsin and collagen (*third series*). Proteases evidently are able to increase hydrophilia in both acid and alkalin media.¹⁵

TABLE 8.

No.	Elastin, Grams	Sodium Carbonate Solution: 1 Per Cent., C.c.	Trypsin, Mg.	Filtrate, C.c.	Amount of Water Absorbed		
					Total, C.c.	Excess over the Control	
						Absolute, C.c.	Relation to the Weight of the Elastin, Per Cent.
1	3	250	None (control)	232	18	—	—
2	3	250	200	226 ¹⁶	24	6	200

THIRD SERIES OF EXPERIMENTS

With collagen and pepsin in 0.025 or 0.05 per cent. hydrochloric acid solution.

9. **Conditions of the ninth experiment:** Same as those of No. 1 (*first series*), except that finely divided collagen (2 grams) from Achilles tendon (oxen) was substituted for fibrin, 0.05 per cent. hydrochloric acid solution (instead of 0.1 per cent.) was used, and the experiment continued six hours. See Table 9.

TABLE 9.

No.	Character of the Mixture			Filtrate, C.c.	Amount of Water Absorbed		
	Collagen, Grams	Hydro- chloric Acid Solution: 0.05 Per Cent., C.c.	Pepsin Solu- tion—0.008 Per Cent., C.c.		Total, C.c.	Excess over the Control	
						Absolute, C.c.	Relation to the Weight of the Collagen, Per Cent.
1	2	250	None (control)	168	82	—	—
2	2	250	0.15	167	83	1	50
3	2	250	0.30	166	84	2	100
4	2	250	0.45	165	85	3	150

10. **Conditions of the tenth experiment:** Same as those of No. 9, except that the concentration of the pepsin product in the

¹⁵ We are now studying the activity of proteases, in this respect, in *neutral* media.

¹⁶ Slight digestion.

enzyme solution was 0.017 per cent. (0.1 gram in 600 c.c.), the strength of the acid was 0.025 per cent., and the duration of the experiment was four hours. See Table 10.

TABLE 10.

No.	Character of the Mixture			Filtrate, C.c.	Amount of Water Absorbed		
	Collagen, Grams	Hydro- chloric Acid Solution; 0.025 Per Cent., Cc.	Pepsin Solution—0.017 Per Cent., C.c.		Total, C.c.	Excess over the Control	
						Absolute, C.c.	Relation to the Weight of the Collagen, Per Cent.
1	2	250	None (control)	152	98	—	—
2	2	250	0.15	151	99	1	50
3	2	250	0.30	145	105	7	350
4	2	250	0.45	148 ¹⁷	102	4	200

The results of the ninth and tenth experiments (with collagen) were especially striking in the differences which were obvious in the heights of the columns of bloated material.

GENERAL DEDUCTIONS

The results of these experiments give added weight to our belief that hydrolases markedly influence the absorption of water by protoplasm.

Our data indicate that both basic and acidic substances may coöperate with proteases in increasing the absorption of water by protein colloids.

These experimental observations also accord with the supposition that edematous changes may result from the action of enzymes independently of acidity or alkalinity.

That the water content of protoplasm is regulated, normally and abnormally, by several important classes of substances, and by more than one direct influence, are fair inferences from the experimental facts at hand.

Studies are now in progress on the influence of enzymes upon the hydrophilic tendencies of tissues in neutral, acid and alkaline media, and in the presence of salins, non-electrolytes and various special substances.

¹⁷ Slight digestion.

STUDIES OF ENZYMES AS POSSIBLE FACTORS IN THE DEVELOPMENT OF EDEMA

3. Is experimental edema in recently excised tissues attended by protein hydrolysis?

EDGAR G. MILLER, JR. AND WILLIAM J. GIES

(Laboratory of Biological Chemistry of Columbia University, at the College of
Physicians and Surgeons, New York)

Introduction. The data in the preceding paper¹ show that small proportions of certain proteases induced marked relative increase in the bulkiness of typical protein masses immersed in various acid and alkaline media. This phenomenon appears to have been brought about by a superficial fixation of water prior to and as a step in the direction of hydrolytic *cleavage*. The results of the same experiments show, also, that comparatively large proportions of proteases in similar alkaline or acid media induced not only *preliminary increases* in the size of some of the protein masses but also *subsequent relative diminutions of bulk*. In such cases the swelling masses were evidently subjected to rapid hydrolytic cleavage, with continuous elision of small and more soluble parts that held, by molecular incorporation, various proportions of the water which at first was superficially combined. "The absorption of water by protoplasm may be influenced to a marked extent by hydrolases," and natural edema may result from, or its development may be modified by, special enzymic fixation of water on tissue colloids, as Tracy and Gies conclude.¹ Nevertheless, the observations of Tracy and Gies also suggest the possibility that special enzymic fixation of water in protoplasm *might be accompanied there by commensurate enzymic hydrolytic cleavage*, normally as well as abnormally, and that, therefore, *natural edema* might never be caused or influenced by hydrolases.²

¹ Tracy and Gies: Biochemical Bulletin, 1912, i, p. 467.

² For the present we ignore the possibility that enzymes, under such circumstances, might increase the total affinity of a part for water by producing in it

Whether such suppositions accord with the facts pertaining to natural edema would seem to depend primarily on the proportions and *activities* of the hydrolases in a given protoplasmic mass. The simplest experiments for direct tests of the matter in question appear to be such as would favor the development of postmortem edema in fresh tissues under definite and controlled conditions. In such experiments the fundamental question would be: Could marked edema occur and *persist* in excised tissues unaccompanied by the formation of special proportions of products of protein hydrolysis?

In the experiments detailed below we have found that this question, so far as it relates to protoplasmic proteins, must be answered in the affirmative. Our data emphasize our conviction that enzymes may be positive factors of importance in the development of edema.³

General procedure. In our experiments in this connection we endeavored to determine, in carefully controlled tests, whether the development of postmortem edema is attended by the production of non-coagulable nitrogenous substances, particularly proteoses and peptones. Surviving tissues from dogs were selected for the experiments. In each case the dog was quickly bled to death painlessly from a femoral artery, after the insertion of a cannula under local cocain anesthesia. Immediately after the death of the animal the warm tissues were removed to weighed closed vessels and the weights of the tissues at once obtained, by difference, prior to the prompt immersion of the tissues in the measured volumes of liquids in reserve to receive them.

Each test in a series was made on two portions of the same kind or mass of tissue from one animal. A first portion of tissue, after its weight had been determined, *was subjected to conditions intended to prevent edema; this was the control tissue.* The weighed second portion of tissue in each test was promptly placed in a moderate excess of water at room temperature for the spontaneous development of edema. After the control portion had been treated for the *prevention* of edema, it was usually subjected to the conditions that prevailed for the second portion. The time allowance for

an increased number of hydrophilic molecules that could not move away from the tissue or which, at all events, would not be removed at the rate of formation.

³ Gies: Biochemical Bulletin, 1911-12, i, pp. 314 and 461.

the development of edema ranged from 24 to 72 hours. An "edema period" of 24 hours was sufficient for our purposes in most of the experiments. Toluene or chloroform, or both, were used as preservatives, when occasion required. The mixtures were gently shaken occasionally during the edema periods.

At intervals during the "edema period," or at its close, the tissue masses were suspended above the open containers, extraneous liquid was allowed to drain back into the main volumes and the weights of the bloated masses were recorded. At the end of each "edema period" the corresponding edematous tissue was subjected to the conditions which had been applied to the control tissue for the prevention of edema. This was done in order to equalize, if possible, any hydrolytic or other disturbing influences that might have been due to the different manipulations.

Promptly after the conclusion of each "edema period," the tissue was minced and then finely triturated with sand in a large mortar. Quantitatively comparable aqueous extracts and filtrates were prepared, under identical conditions in each series of tests. The mixtures were thoroughly shaken repeatedly during the "extraction periods."

Aliquot portions of the neutralized filtrates were carefully freed from coagulable protein by the heat coagulation process.⁴ "Non-coagulable nitrogen" in the filtrates was determined by the Kjeldahl method.

The special details of each series of experiments are stated in the ensuing descriptions. The analytic data are summarized in the table on page 480.

Experiments. *First series: With kidneys.* The control kidney was placed in 100 c.c. of water + 100 c.c. of 95 per cent. alcohol. The second kidney was immersed in 100 c.c. of water. It was assumed that the alcohol would prevent appreciable hydrolysis in the control kidney. These conditions were maintained for 24 hours. At the end of that time, 100 c.c. of 95 per cent. alcohol were added to the liquid containing the *edematous* kidney. The extraction volumes were then equal. The new conditions prevailed

⁴We added dilute acetic acid solution, little by little *while the liquid was boiling* (not before), in order to effect complete coagulation.

from the beginning of the 25th to the 72d hours, inclusive. At the end of this period the alcoholic liquids were poured off and retained. In each case the macerated tissue was further extracted for 48 hours with 500 c.c. of 5 per cent. sodium chloride solution. The alcoholic and saline extracts from each tissue were combined before the analytic procedure was started.

*Second series: With kidneys, testicles and heart.*⁵ The tissues were subjected to conditions which were essentially the same as those that prevailed in the first experiment, but 20 per cent. sodium chloride solution was used instead of 95 per cent. alcohol in the preliminary treatment and instead of 5 per cent. sodium chloride solution for the final extractions.

Third series: With kidneys and heart. The results of the foregoing tests (see the data on page 480) suggested that, in all but one instance, sufficient hydrolysis of protein occurred in the edematous tissues to give them perceptibly larger contents of non-coagulable nitrogenous substances. We then assumed that our treatment for the total inhibition of enzymic hydrolysis in the control tissues had not been wholly successful in the first two series of tests, and concluded that the observed difference between the corresponding amounts of "non-coagulable nitrogen" would be greater if enzymic cleavage were completely prevented in new *control* masses. In this series of tests we accordingly aimed to *destroy* all enzymes in the control tissue, with the aid of heat but without causing hydrolysis by the heating process.

The control masses were dropped into *boiling* 95 per cent. alcohol⁶ in covered beakers on water baths. The boiling was continued for six minutes. After the alcoholic extract became cold (an hour later), the tissue was kept in 150 c.c. of water about 24 hours. For the second portion of each tissue ("edema tissue"), the order

⁵In this series and in all others that included experiments on heart, the organ was divided longitudinally through the auricles and ventricles into approximately equal masses, one of which was used for control purposes, the other for the edema tests.

⁶We selected alcohol for this purpose because of its *dehydrating* influence. At the temperature of boiling alcohol under the imposed conditions, the tissue enzymes must have been destroyed, the coagulable proteins were coagulated and little if any hydrolysis could have taken place.

of treatment was reversed—24 hours in water, six minutes in boiling alcohol and one hour in the latter as it cooled.

At the end of the "edema period" the triturated tissue in each case was placed in 200 c.c. of water and aqueous extraction was continued for 24 hours. The corresponding extracts were combined before analysis was begun.

Fourth series: With kidneys and heart. The results of the third series of tests did not bear out the assumption on which the special treatment was based. To our surprise the edematous tissues yielded smaller quantities of "non-coagulable nitrogen" than the controls. At this point our table showed three plus balances and three minus balances.⁷ It was then assumed that the production of non-coagulable protein would increase greatly during the period of shrinkage and softening that follows the attainment of the maximum degree of bloating in such experiments. In the fourth series of tests we accordingly duplicated the conditions of the third, except that the "edema period" was 72 hours instead of 24 hours, and the water surrounding the tissues was renewed at the end of each of the three days.⁸ All the corresponding extracts were combined before the analyses were begun.

The result of the test on kidney is striking. The edematous kidney gained a fairly large proportion of "non-coagulable nitrogen." *This particular mass of edematous tissue was the only one (IV-V) that lost considerable weight after a period of 24 hours.* Possibly this loss of weight was due in part to enzymic formation,

⁷ Every endeavor was made to prevent analytic error or irregularities in duplicate processes. It is possible that the *preliminary* treatment of the control tissue with hot alcohol in the third series was more effective in preparing the tissue for complete extraction than the similar treatment of the edematous tissue *at the end of the experiment*. The control tissue could be more finely triturated because, even after the concluding alcohol treatment of the edematous tissue, the latter was softer, apparently more hydrous, and probably more retentive of extractable substances. We are fully aware, also, of the difficulties besetting quantitative removal of such substances as proteoses and peptones from tissues. For these reasons we do not lay much stress on the differences for "non-coagulable nitrogen" in any of the tests of the first three series of experiments.

⁸ This course of treatment prevented cumulative increase of an edema-favoring acidity, but it also effected the removal of such inhibitive factors as salins.

and the elimination, of diffusible colloid cleavage products, with a consequent reduction of total hydrophilic power.

Fifth series: With leg muscle. The conditions of this experiment were practically the same as those for the fourth series of tests. The volumes of alcohol⁹ and water were 500 c.c.

Data showing especially the amounts of non-coagulable nitrogen from edematous tissues and from corresponding control tissues which were prevented from becoming bloated

Series No.	Fresh Tissue					"Non-coagulable Nitrogen" in the Extracts*			
	Kind	Weight, Grams	Percentage Gain in Weight of Edematous Tissue			Total Amount Found, Mg.	Per 100 Grams of Fresh Tissue		
			24 Hr.	48 Hr.	72 Hr.		Total Calculated, Mg.	Gain or Loss over the Control	
								Gain, Mg.	Loss, Mg.
I. §	Kidney : c †	19.9				105	526		
	e	18.5	59.4	108	582	56	
II. §	Kidney : c	32.0				144	449		
	e	31.2	44.2	171	547	98	
	Testicle : c	18.0				65	366		
	e	18.0	10.5	81	453	87	
	Heart : c	53.9				191	354		
	e	57.7	14.9	197	342	...	12
III. §	Kidney : c	22.9				125	547		
	e	23.5	91.9	102	434	...	113
	Heart : c	36.6				80	215		
	e	41.4	20.7	67	161	...	54
IV.	Kidney : c	19.5				104	534		
	e	18.5	63.2	56.7	47.5	137	743	209	
	Heart : c	20.3				78	387		
	e	16.6	27.1	29.5	26.5	71	427	40	
V.	Muscle : c	267.2				668	250		
	e	328.3	19.0	15.8	1,001	305	55	

⁹ Preliminary treatment with hot alcohol in these experiments (III-V) caused hardening and decided loss of weight. Immersion in water afterward, for three days or more, failed with one exception to restore the original weight of the tissue.

* We determined the *total nitrogen* in each extract with intention to express our "non-coagulable nitrogen" values in terms of their proportion of the total nitrogen in the corresponding extracts. We abandoned this purpose after it became evident that the figures for total nitrogen in the extracts did not provide a reliable basis for the comparisons, although the data thus obtained warranted the general conclusions we have drawn from the figures in this table.

§ The "edema period" for each of the first three series was 24 hours.

† The letters c and e indicate respectively the *control* and *edematous* portions. In the table the data for the edematous portions are printed in heavy-faced figures.

|| The "edema period" for the last two series was 72 hours.

Discussion of results. Our results show that when various types of surviving tissues from dogs (kidney, heart, leg muscle and testicle) are immersed in water at room temperature, *for periods of 24 hours*, they become markedly edematous but do not acquire much larger proportions of non-coagulable nitrogenous products than those contained in control non-edematous tissues under similar experimental conditions. The data for kidney in the fourth series, when viewed in the light of all the associated results, suggest that comparatively little non-coagulable nitrogenous material was formed in any of the tissues during the *development* of edema (*i. e.*, comparatively little hydrolytic *cleavage* occurred), *before the tissue began to lose water as a result of dissolutive (enzymic?) influences*. Many more experiments must be performed before it can be asserted that this is the rule in such cases.

Our results do not demonstrate that enzymes were involved to any extent in the development of the observed edemas. The tests were directed at negative phases of that question. Our data prove, however, that *extensive* edema may occur progressively under the conditions of these experiments, for 24 hours or longer, before very marked hydrolytic (autolytic) *cleavage* of protein takes place. The results obtained by Tracy and Gies, supported by the data in this paper, warrant the opinion, we think, that, under certain conditions, enzymes may be important *positive* factors in the development of edema without inducing commensurate or even appreciable dissolution, by hydrolysis or any other process. In our view of the rôle of enzymes as positive factors in the development of edema, the enzymes may cause fixation of water on a given insoluble mass or in a tissue without forcing the affixed water, or any available free water, to disintegrate or disorganize the material by entering into (and disrupting) any of its molecules, *i. e.*, without inducing appreciable hydrolytic *cleavage*. In *natural edema* the proportion of *active* (effective) hydrolase in any tissue may be determined or *regulated* by a number of associated influences but, while stimulative of hydrophilic tendencies, may not be sufficient to bring about special hydrolytic *cleavage* at any time, thus permitting, or accounting for, the attainment of maximum edematous

effects. On the other hand, the natural prevention or resolution of edema in any part under certain conditions might be facilitated by disturbances of equilibria which would remove restraint from, or which would accelerate, the hydrolytic action of effective proportions of hydrolases.

These views are tentative conceptions. They afford a basis for the work in this field which is now in progress, in a number of the relations that are suggested by the recorded data.

A NOTE ON THE DISTRIBUTION OF CHLORATE IN A WOMAN FATALLY POISONED BY POTASSIUM CHLORATE¹

JACOB ROSENBLOOM

(Laboratory of Biological Chemistry of Columbia University, at the College
of Physicians and Surgeons, New York)

Through the kindness of Prof. J. H. Larkin, the writer received certain organs from a woman who had taken about 50 grams of potassium chlorate by mistake. It was thought that a study of the amount of potassium chlorate in these organs would prove of interest, especially as *the analysis could be started within twelve hours after the woman's death.*

The materials received for examination, together with the amounts of potassium chlorate found in each, are indicated below:

Material	Amount	Potassium Chlorate	
		Found, Gram	Total Amount Recovered, Gram
Intestinal contents.....	320 c.c.	0.0393	
Kidney.....	55 grams	0.0177	
Spleen.....	82 "	0.0922	
Liver.....	162 "	0.0854	
Blood (from liver).....	200 "	0.0623	
Heart.....	42 "	0.0495	0.3464

The chlorate was determined by the usual method of precipitating aliquot portions with silver nitrate before and after reduction with zinc dust, and weighing the silver chloride precipitates. The writer found that such precipitates are often contaminated with purin bases. They must be dissolved in warm ammonium hydroxid solution and reprecipitated with nitric acid for the purification of the silver compound.

Since this woman took about 50 grams of potassium chlorate, the rapidity of the excretion of the salt and of the reduction of the chlorate to chloride are well shown by the smallness of the quantities present in the tissues, as chlorate, at the time the analysis was made.

¹ Prof. John H. Larkin will publish a pathological study of the organs of this case at an early date. For a thorough discussion of potassium chlorate poisoning see Witthaus: Toxicology, 1911, p. 690.

THE BIOCHEMICAL CLUB, ENGLAND

This association has been instituted for the purpose of facilitating the intercourse between those biologists and chemists who are interested in the investigations of problems common to both, such as the chemical problems connected with agriculture, brewing, animal and vegetable physiology, and pathology.

It was founded at an informal meeting held in London on Jan. 21, 1911, which was arranged by Drs. J. A. Gardner, and R. H. Aders Plimmer, and at which about fifty workers in biological chemistry were present. A provisional committee consisting of Dr. J. A. Gardner, Dr. A. E. Garrod, F.R.S., Professor W. D. Halliburton, F.R.S., Dr. E. J. Russell and Dr. R. H. Aders Plimmer (Secretary) was elected.

After a preliminary meeting at which the rules for the Club were drawn up, meetings of a scientific nature have been held at the University College, London; the Physiological Laboratory, Oxford; the Rothamstead Experimental Station; the City and Guilds (Engineering) College; the School of Agriculture, Cambridge; King's College, London; the Lister Institute, London; and St. Bartholomew's Hospital, London.

The meetings are quite informal; papers are read and demonstrations given, and free discussion indulged in. As the proceedings are not published, there is opportunity for free and frank interchange of views. At the conclusion of each scientific meeting, the members of the Club dine together.

The programme for the present year includes meetings at University College, Reading; the Rose-Innes Institute, Wimbledon; the Woburn Fruit Farm; Guy's Hospital, London; the University of Birmingham; the Cancer Hospital, London; and the London Hospital.

The present committee consists of Professor H. E. Armstrong, F.R.S., Dr. W. M. Bayliss, F.R.S., Mr. J. S. Ford, Dr. H. H. Dale, Dr. J. A. Gardner, Dr. W. H. Hurtley, Dr. A. Harden, F.R.S., Dr. F. G. Hopkins, F.R.S., Mr. F. Keeble, Professor B. Moore, F.R.S.,

Dr. W. Ramsden, Mr. E. J. Russell, Professor J. Lorrain Smith, F.R.S., and Dr. R. H. Aders Plimmer (Treasurer and Secretary).

Some changes are contemplated in the constitution of the Club during the ensuing year. For instance, its title may possibly be the Biochemical Society, instead of Club, although the proceedings at the meetings will still remain of the pleasant informal character as heretofore. Negotiations are also in progress for the purchase of the *Bio-Chemical Journal*, hitherto published at Liverpool under the editorship of Professor B. Moore, and Dr. Whitley. This will then be the organ of the Society, and it is hoped that in this way the usefulness and circulation of that Journal will be increased. A further note will, however, be sent to the BIOCHEMICAL BULLETIN on this subject when the matter is finally settled.

The foundation of this association is to be regarded as a step in the right direction; the meetings hitherto have been stimulating and full of interest, and there can be no doubt that the coöperation of workers in different fields of bio-chemistry will advance the progress of that science.

W. D. H.

BIOCHEMICAL NEWS, NOTES AND COMMENT

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I. GENERAL

Necrology. Dr. Théophile Durand, director of the Jardin Botanique de l'Etat at Brussels.

Dr. Ditmar Finkler, retired professor of hygiene at the University of Bonn.

Dr. Paul G. Freer, director of the United States Government Scientific Bureau in the Philippines and editor of the *Philippine Journal of Science*.

Dr. G. Armauer Hansen, the Norwegian biologist and zoologist, and the discoverer of the bacillus of leprosy (1871).

Dr. Waldemar Koch, assistant professor of pharmacology in the University of Chicago. (See pages 372 and 522.)

Dr. Joseph Lister, founder of the antiseptic method in surgery. (See pages 371, 487 and 519.)

Dr. Naokichi Matsui, Dean of the Agricultural College of the Imperial University of Tokyo.

Enno Sander, a well-known pharmacist of St. Louis, a charter member of the Association of Military Surgeons of the United States, and a pharmaceutical member of the American Medical Association.

In memoriam. *Lord Lister.* The lord provost of Glasgow proposes to convene a conference to promote an international memorial to Lord Lister in Glasgow. (See pages 371, 487 and 519.)

At a meeting of the council of the Royal College of Surgeons, Sir Henry Morris, past president and senior member, moved a vote of condolence to the family of the late Lord Lister, to whose work he paid the following eloquent tribute:

Surgery has lost her most brilliant student and her greatest master, England one of her most famous sons, the world one of its most illustrious citizens. He raised surgery from a dangerous and precarious practice to a precise, safe and beneficent art. His methods have been adopted in every country and the benefits which flow from them are blessings conferred on every race of mankind. His perspicacity, his natural insight, his fertility of resource, his power of close and discriminating observation, his philosophic reasoning, his inflexible pursuit of truth, his steadfastness of purpose, his capacity for taking pains, his unwearied patience and his undaunted efforts to triumph over difficulties, stamped him as a great example of true and scientific genius. The human sympathy which caused him to deplore the great mortality due to infective surgical disease, his solicitude to prevent suffering and premature death, his patient and unceasing labor to overcome them, his gratification at the ultimate success of his efforts to ameliorate pain and prolong life, eminently distinguish him as a great philanthropist. His gentle nature, his deep compassion, his courteous and dignified bearing, his imperturbable temper, his resolute will, his indifference to ridicule, his tolerance of hostile criticism, combined to make him one of the noblest of men. His work will last for all time; its good results will continue throughout all ages; humanity will bless him forevermore; his fame will be immortal. In the fulness of years, after a glorious and satisfying career, crowned with great and various honors, having witnessed the successful completion of his work, the gentle hand of Death laid hold on him and Lister passed from among us. His body perisheth, but the influence of his mind will continue everlastingly.

It was resolved that this memorial of esteem and admiration be printed, surrounded by the symbol of mourning, in the college minutes and that an inscribed tablet should be placed in a suitable and conspicuous position within the walls of the college, to serve as evidence to future generations of the honor, respect, and reverence in which the great founder of aseptic surgery was held.

Under the terms of his will, Lord Lister requested his nephews, Mr. R. J. Godlee, president of the Royal College of Surgeons, and

Dr. A. H. Lister, to arrange his scientific manuscripts and sketches, destroying or otherwise disposing of such as are of no permanent interest. He bequeathed the said manuscripts and sketches, when so arranged, to the Royal College of Surgeons. (See page 503.)

Crawford W. Long. On March 30, 1842, in the village of Jefferson, Georgia, Dr. Crawford W. Long administered ether to Mr. James Venable and, while the patient was completely anesthetized, removed a small tumor from the back of his neck. On the seventieth anniversary of the day, exercises in honor of Long were held in the Medical School of the University of Pennsylvania, from which he graduated in 1839. Addresses were made by Prof. J. William White, of the University of Pennsylvania, and by Prof. J. Chalmers Da Costa, of the Jefferson Medical College. A bronze medallion designed by Prof. R. Tait Mackenzie, of the University of Pennsylvania, was unveiled by one of the three daughters of Dr. Long, who were present at the ceremony. Besides the unveiling of the tablet to the memory of Long, on March 30, additional honor was paid to his memory, when a portrait of Long, painted by one of his daughters, was unveiled on April 1 with appropriate ceremonies in the medical school building.

H. P. Bowditch. At the recent meeting of the American Physiological Society in Baltimore, Prof. W. B. Cannon, of the Harvard Medical School, delivered a memorial address on the late Prof. Henry Pickering Bowditch.

Sir Michael Foster. Dr. J. B. Hurry has established a research studentship of physiology at Cambridge to be named in honor of Michael Foster.

Mrs. Ellen H. Richards. At an important meeting of the Ellen H. Richards Memorial Fund Committee, held January 20, 1912, the fund was specifically designated the Ellen H. Richards Home Economics Fund, and its object defined as the application of the results of scientific investigation for advancing the interest of the home. The immediate purpose, as decided, is to establish permanently the *Journal of Home Economics*, the one scientific journal devoted to advanced housekeeping, and upon which Mrs. Richards was engaged at the time of her death; other objects contem-

plated are investigation, publication, fellowships, lectureships, etc. The selection of permanent trustees was considered and plans outlined for an active campaign for soliciting funds which is now under full headway. Mrs. William H. Barrett, 108 Johnson St., Brooklyn, N. Y., is chairman of the committee, and Dr. Benjamin R. Andrews, Teachers College, New York City, is its secretary-treasurer. (See page 346.)

Honors. *Newly elected members of national societies.* Sir J. J. Thomson has been appointed by King George V a member of the Order of Merit. The other scientific men who are members of the order are Lord Rayleigh, Dr. A. R. Wallace and Sir William Crookes. The order has recently lost through death Sir Joseph Dalton Hooker and Lord Lister.

Prof. Benjamin Moore is one of fifteen candidates officially nominated recently for membership in the Royal Society.

Among the newly elected members of the National Academy of Sciences are Prof. John J. Abel, Dr. Charles B. Davenport and Dr. S. J. Meltzer.

Newly elected members of foreign societies. Professor Ehrlich has been elected an honorary member of the Belgium Academy of Medicine.

Professor Hermann, the physiologist of Königsberg, and Prof. A. Kossel, of Heidelberg, have been elected to honorary membership in the Belgium Academy of Sciences.

Prof. E. Metchnikoff, assistant director of the Pasteur Institute at Paris, has been elected foreign associate of the French Academy of Sciences, in succession to Sir Joseph Hooker. At the anniversary meeting of the Royal Irish Academy, he was elected an honorary member of the academy in the section of science.

Sir J. J. Thomson has been elected a foreign member of the Naples Academy of Sciences.

Awards of medals. At the meeting of the Perkin Medal Committee (consisting of representatives of the Society of Chemical Industry, the American Chemical Society and the American Electrochemical Society), held on December 15, the Perkin medal for 1912 was unanimously awarded to Mr. Herman Frasch for his chemical engineering work in the sulphur and oil refining industries.

Prof. Charles James, of New Hampshire College, has been awarded the Nichols medal of the American Chemical Society.

Professor Rubner, director of the Berlin hygienic institute, has received the large gold Rinecker medal of the University of Würzburg.

Prize. The Academy of Sciences of the Royal Institute of Bologna has awarded the Élie de Cyon prize of 3,000 lire for 1911 to Prof. E. A. Schäfer, F.R.S., of Edinburgh, in recognition of his work on the ductless glands and especially his recent work on the pituitary body.

Dinners. A dinner was given to Prof. Ludvig Hektoen at the Chicago Club, on April 11, by the faculties of Rush Medical College and the College of Physicians and Surgeons, and by Professor Hektoen's former students at these institutions, in honor of the twenty-first anniversary of his entrance into the practise of medicine. Dr. Frank Billings presided. An oil-painting of Dr. Hektoen was presented to him by his friends, the presentation speech being made by Dr. E. R. Le Count. Prof. E. O. Jordan and Drs. H. Gideon Wells and James B. Herrick responded to toasts.

Prof. Alexander Smith was the guest of the Alumni of the Schools of Applied Science of Columbia University at a dinner held on the evening of January 25, at the Chemists' Club, New York. Profs. Charles F. Chandler and R. H. Chittenden were among the speakers.

The evening of January 23 was "Doctors' Evening" at the University Club in Brooklyn and Dr. Harvey W. Wiley was the guest of honor. Among the other speakers were Dr. William M. Polk, dean of the Cornell Medical College; Dr. Eugene H. Porter, formerly state health commissioner; Dr. Edward E. Hicks, Brooklyn; Dr. Elias H. Bartley, Brooklyn, of the Kings County Medical College; and Zachary T. Emery, formerly health commissioner of Brooklyn.

Birthday celebration. The pupils and friends of Professor Ludwig, lecturer on medical chemistry at Vienna University, recently celebrated his seventieth birthday. For a number of years Professor Ludwig has been one of the most esteemed and beloved

teachers in Vienna. His pupils hold important positions all over Austria and Germany, and he is himself one of the chief personalities of the Austrian board of health, which has become an important international organization through his perseverance. Forensic chemistry has been brought to such a high point by his endeavors that this alone would suffice to make his name widely known in the scientific world.

Honorary degrees. At the University of Pennsylvania's celebration of Washington's birthday, the doctorate of public hygiene was conferred on Dr. A. C. Abbott.

Among the recipients of honorary degrees, on the occasion of the exercises commemorating the one hundred and twenty-fifth anniversary of the establishment of the University of Pittsburgh, were Drs. N. L. Britton, James Ewing, L. O. Howard and Edgar F. Smith.

Portrait presented. A portrait of Dr. James Tyson, emeritus professor of medicine, painted by Mr. Hugh H. Breckenridge, has been presented to the University of Pennsylvania.

Resignations. Dr. Henry A. Christian has resigned as dean of Harvard Medical School, to take effect September 1, 1912, and as physician-in-chief of the Carney Hospital, to take effect June 1, 1912. (See page 500.)

Dr. Raymond A. Pearson has resigned the office of Commissioner of Agriculture of the state of New York. (See page 492.)

Dr. Ira Remsen has tendered his resignation as president of Johns Hopkins University. He will remain professor of chemistry.

Dr. William Trelease has resigned the directorship of the Missouri Botanical Garden. (See page 492.)

Dr. H. W. Wiley has resigned the position of Chief of the Bureau of Chemistry of the U. S. Department of Agriculture. (See pages 391 and 505.)

Appointments. Recent appointments to the research staff of the Otho S. A. Sprague Memorial Institute, Chicago, are Dr. Samuel Amberg, of the department of pediatrics at Johns Hopkins Medical School, and Dr. Lydia M. DeWitt, formerly of the department of anatomy at the University of Michigan, and more recently of the health department of the city of St. Louis.

Dr. Horace David Arnold has been appointed dean of the Graduate School of Medicine of Harvard University. The graduate school of medicine is a new department of the university, and is under the jurisdiction of the faculty of medicine. (See page 500.)

Frederick H. Blodgett, Ph.D. (Hopkins, '10), acting professor of biology and geology, has resigned from Roanoke College and assumed the duties of plant pathologist and physiologist at the Texas Experiment Station, College Station, Texas, on February 1. The work interrupted by the sudden death of Dr. Raymond H. Pond last summer (pages 146 and 354) will be resumed and some additional attention paid to plant diseases.

Dr. Rupert Blue, of South Carolina, has been appointed surgeon general of the public health and marine hospital service, succeeding the late Dr. Walter Wyman.

Dr. Fingerling, of the Agricultural School at Hohenheim, has been elected director of the Agricultural Experiment Station at Möckern.

Dr. C. N. Jensen, fellow in plant pathology, Cornell University, has been appointed professor of botany and plant pathology in Utah Agricultural College and Experiment Station.

Dr. George T. Moore, professor of plant physiology at the Shaw School of Botany, and until recently plant physiologist at the Missouri Botanical Garden, has been appointed director of the Garden to succeed Dr. William Trelease.

A bacteriological department of the Berlin pathological institute has been formed, and Professor Morgenroth, a pupil of Ehrlich, has been named as its director.

Dr. Raymond A. Pearson, recently commissioner of agriculture for the state of New York, has accepted the presidency of the Iowa State College of Agriculture at Ames.

Dr. C. Raunkiär has been appointed professor of botany and director of the botanical garden at Copenhagen in succession to Professor Eugene Warming, who retires from active service.

Dr. J. N. Rose has been appointed research associate in the department of botanical research of the Carnegie Institution.

Dr. L. A. Ryan has been appointed assistant professor of chem-

istry and toxicology in the medical school of the University of Pennsylvania.

Regierungsrat Dr. Weber, who aided in the scientific organization of the Dresden International Hygiene Exposition, has been appointed director of the imperial health office (Gesundheitsamt) to succeed Professor Uhlenhuth, who is at present director of the hygienic institute at Strasburg.

Dr. Lilian Welsh, professor of physiology and hygiene in Goucher College, has been appointed special lecturer on hygiene to the women students of the University of Michigan. (See page 504.)

Prof. H. Staudinger, of the Technische Hochschule, Karlsruhe, has been appointed to the chair of organic chemistry at the Eidgenössische Technische Hochschule, Zürich, to succeed Prof. Richard Willstätter, who will direct the work in organic chemistry of the new Kaiser Wilhelm Research Institute at Berlin.

Officers-elect of biological societies. The names of officers elected at the last annual meetings of leading biological societies are grouped below:

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS: *President*, A. B. Macallum; *Vice-President*, Graham Lusk; *Secretary*, A. N. Richards; *Treasurer*, Walter Jones; *Additional members of the Council*: H. P. Armsby, Lafayette B. Mendel and H. Gideon Wells; *Nominating Committee*: J. J. Abel, F. G. Benedict, H. C. Bradley, Otto Folin, William J. Gies, Andrew Hunter, J. B. Leathes, J. J. R. Macleod, D. D. Van Slyke; *Committee on nomenclature and classification of fats and fat-like substances*: Waldemar Koch, H. D. Dakin, William J. Gies, J. B. Leathes and Jacques Loeb.

AMERICAN CHEMICAL SOCIETY: *President*, A. D. Little; *Secretary*, Chas. L. Parsons; *Treasurer*, A. P. Hallock.

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE: *President*, James Ewing; *Vice-President*, P. A. Levene; *Secretary*, George B. Wallace; *Treasurer*, Charles Norris.

AMERICAN HOME ECONOMICS ASSOCIATION: *President*, Isabel Bevier; *Vice-Presidents*, C. F. Langworthy, Martha Van Rensselaer, Abby L. Marlatt; *Secretary*, B. R. Andrews; *Treasurer*, Howard L. Knight; *Councillors* (five years), Ellen A. Huntington, Catherine

J. McKay, Louise Stanley, Mary L. Tuttle, Mrs. Mary P. Van Zile; Agnes Harris vice Abby L. Marlatt and Caroline L. Hunt vice Mrs. Ellen H. Richards.

AMERICAN SOCIETY OF NATURALISTS: *President*, E. G. Conklin; *Vice-President*, R. G. Harrison; *Secretary*, A. L. Treadwell; *Treasurer*, W. E. Kellicott; *Additional members of the Executive Committee*: B. M. Davis and H. E. Jordan.

AMERICAN SOCIETY OF ZOOLOGISTS—EASTERN BRANCH: *President*, A. G. Mayer; *Vice-President*, G. A. Drew; *Secretary-treasurer*, John H. Gerould; *Additional members of the Executive Committee*: David H. Tennent, Ross G. Harrison and H. E. Jordan.—CENTRAL BRANCH: *President*, H. B. Ward; *Vice-President*, C. M. Child; *Secretary-treasurer*, W. C. Curtis; *Additional members of the Executive Committee*: C. E. McClung (3 yrs.), H. F. Nachtrieb (2 yrs.).

BOTANICAL SOCIETY OF AMERICA: *President*, L. R. Jones; *Vice-President*, B. M. Duggar; *Secretary*, G. T. Moore; *Treasurer*, Arthur Hollick; *Councillors*: C. L. Shear, R. A. Harper and Wm. Trelease.

AMERICAN PHYSIOLOGICAL SOCIETY: *President*, S. J. Meltzer; *Secretary*, A. J. Carlson; *Treasurer*, W. B. Cannon; *Additional members of the Council*: Joseph Erlanger and Frederic S. Lee.

SECTION (K) OF PHYSIOLOGY AND EXPERIMENTAL MEDICINE OF THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE: *Vice-President*, J. J. R. Macleod; *Secretary*, Waldemar Koch.

AMERICAN SOCIETY FOR PHARMACOLOGY AND THERAPEUTICS: *President*, John J. Abel; *Secretary*, John Auer; *Treasurer*, A. S. Loevenhart; *Additional members of the Council*: Reid Hunt and G. B. Wallace.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS: *President*, Herbert U. Williams; *Vice-President*, J. J. MacKenzie; *Secretary*, H. C. Ernst; *Treasurer*, F. B. Mallory; *Councillors*: W. H. Park, Leo Loeb and E. R. Le Count.

SOCIETY OF AMERICAN BACTERIOLOGISTS: *President*, William H. Park; *Vice-President*, C.-E. A. Winslow; *Secretary-treasurer*, Charles E. Marshall; *Councillors*: W. J. MacNeal, Otto Rahn, H. D. Pease, John F. Anderson.

AMERICAN INSTITUTE OF CHEMICAL ENGINEERS: *President*, L. H. Baekeland; *Vice-Presidents*, Eugene Haanel, T. B. Wagner, M. C. Whittaker; *Secretary*, John C. Olsen; *Treasurer*, F. W. Frerichs; *Auditor*, G. W. Thompson; *Directors*: A. C. Langmuir, H. S. Miner and A. Bement.

HARVEY MEDICAL SOCIETY: *President*, Frederic S. Lee; *Vice-President*, Wm. H. Park; *Treasurer*, Edward K. Dunham; *Secretary*, Haven Emerson; *Additional members of the Executive Committee*: S. J. Meltzer, Graham Lusk, W. G. MacCallum.

AMERICAN ASSOCIATION FOR CANCER RESEARCH: *President*, E. E. Tyzzer; *Vice-President*, Leo Loeb; *Secretary*, S. B. Wolbach.

MISCELLANEOUS. Colonel Wm. P. Gorgas has been elected *president* of the Ninth Congress of American Physicians and Surgeons, which meets in Washington in May, 1913.

M. Lippman has been elected *president*, and Professor Guyon *vice-president* of the Paris Academy of Sciences.

Prof. Edward C. Pickering has been elected *president* of the American Association for the Advancement of Science.

Dr. Victor C. Vaughan *presided* at the annual Michigan Health Officers' Convention, held in Ann Arbor, January 30-31.

Dr. W. D. Bigelow was elected *councillor-at-large* (for three years) of the American Chemical Society.

Special committees. *National committee for mental hygiene.* The National Committee for Mental Hygiene has received an anonymous gift of \$50,000, which enables the committee to proceed effectively with its plans to improve the conditions affecting the mentally afflicted. At its fourth annual meeting in New York, on February 17, the committee elected officers, among them Lewellys F. Barker, president; William H. Welch, vice-president; George Blumer, chairman of the executive committee; Russell H. Chittenden, chairman of the finance committee.

Commission on resuscitation. At the request of the National Electric Light Association, a Commission on Resuscitation has been organized. Its chairman is Prof. Walter B. Cannon. Drs. Yandell Henderson, George W. Crile and S. J. Meltzer represent the American Medical Association. Prof. E. A. Spitzka is a member

of the commission by nomination of the National Electric Light Association.

Commission on typhoid fever. Commissioner Lederle, of New York City, has appointed a commission of physicians and sanitary experts to study the methods of the local Health Department in handling typhoid and to suggest, if possible, improved methods. Among those who were present at the first meeting of the commission were Prof. William T. Sedgwick, Prof. George Whipple, Prof. C.-E. A. Winslow, Dr. John Winters Brannan, Dr. Alexander Lambert, Dr. Herbert D. Pease, and a number of representatives of the New York Health Department.

Advisory board of health. Dr. Joseph J. O'Connell, health officer of the port of New York, has obtained the coöperation of an advisory board consisting of Drs. James Ewing, professor of pathology in Cornell University Medical College; Dr. John H. Larkin, associate professor of pathology in the College of Physicians and Surgeons; Dr. Joshua M. Van Cott, professor of pathology and bacteriology in Long Island College Hospital, and Dr. Francis Carter Wood, head of the department of clinical pathology in the College of Physicians and Surgeons. This advisory board will be in complete control of the bacteriological and pathological department of the port, on which the safety of the port against epidemic diseases depends to a very large extent.

American commissions on chemical nomenclature.—*Inorganic nomenclature:* James L. Howe, chairman; P. E. Browning, E. C. Franklin, A. M. Patterson, Charles H. Herty, Owen Shinn and Adolf Law Voge.—*Organic nomenclature:* M. T. Bogert, chairman; Ira Remsen, W. A. Noyes, T. B. Johnson, J. B. Tingle, J. F. Norris, M. Gomberg and C. S. Hudson. Both commissions will coöperate with similar national bodies in other countries in the revision of chemical nomenclature. The chairmen will be glad to receive suggestions from American chemists.

American Association of Refrigeration: Committee on state and national investigations. Mary E. Pennington, chairman; A. V. Staubenrauch, W. A. Stocking, Jr., R. M. Allen and H. J. Eustace. The Third International Congress of Refrigeration will be held in Chicago, in September, 1913.

Society notes. The *American Society of Biological Chemists* held its sixth annual meeting in two general sessions: one, at Baltimore, December 27-28, in affiliation with the American Physiological Society and the Society for Pharmacology and Experimental Therapeutics; the other, at Washington, December 29, in affiliation with the Biological Section of the American Chemical Society. No. 2 of volume II of the *Proceedings of the American Society of Biological Chemists* gives an account of the transactions of these sessions. Abstracts of papers also appear in the *Journal of Biological Chemistry* (xi, March), the *American Journal of Physiology* (xxix, February), and the *Journal of Pharmacology and Experimental Therapeutics* (iii, March).

A general account of the proceedings of the Washington meeting of the *American Association for the Advancement of Science* (December 27-31, 1911) was published in the January 5 issue of *Science* (34, 1-8). The next meeting of the American Association will be held at Cleveland, Ohio, beginning on Monday, December 30, 1912.

At the last annual meeting of the *American Chemical Society* the Biological Section of the Society was authorized to form a *Biological Division*. The officers of the Biological Section were: Chairman, Carl L. Alsberg; Secretary, Isaac K. Phelps. It was also voted to donate the library of the American Chemical Society to the New York Chemist's Club on condition that members of the Chemical Society have ready access thereto.

A movement to unite all American botanical associations under the *Botanical Society of America* was auspiciously inaugurated at Washington during Convocation Week.

Lectures. *Cutter lecture.* The first of the annual series of lectures was given on the Cutter foundation at Harvard Medical School, March 13, by Dr. William H. Park, on "Observations on dosage and methods of injecting antitoxin in the treatment and prevention of diphtheria and tetanus."

The *Norman W. Harris lectures* of Northwestern University were given, April 15 to 20, by Dr. Milton J. Rosenau, on the general subject of "Milk and its Relation to Public Health." The succes-

sive lectures dealt with "Dirty milk," "Diseases spread by milk," "Clean milk," "Pasteurization" and "From cow to consumer."

Harvey lectures were delivered on February 3 by Prof. T. W. Richards, on "The relations of modern chemistry to medicine"; on February 17 by Prof. R. H. Chittenden, on "Current views regarding the nutrition of man"; on March 3 by Prof. H. S. Jennings, on "Old age, death and the meaning of conjugation in lower animals"; and on March 23 by Prof. W. S. Thayer, on "Malaria."

Herter lectures. Prof. Ludvig Hektoen delivered six Herter Foundation lectures, under the auspices of the University and Bellevue Hospital Medical College, in the Carnegie building, at 4 o'clock in the afternoon, from January 8 to 13, inclusive. The subject of the lectures was "Immunity."

Hitchcock lectures. Prof. Richard M. Pearce delivered five lectures on the Hitchcock Foundation, at the University of California, on the evenings of January 22-26, inclusive. The general theme of the series was "Research in medicine" and the individual lectures were devoted to the following subjects: "Antiquity to 1800—the efforts of isolated investigators," "The development of laboratories for the medical sciences," "Pasteur and the rise of bacteriology," "Present day methods and problems," and "Medical research in American universities; present facilities, needs and opportunities."

The fifth of the *Weir Mitchell lectures* of the College of Physicians, Philadelphia, was delivered on March 29, in Mitchell Hall, by Prof. William H. Howell, on "The factors concerned in the coagulation of blood and their variations under pathologic conditions."

Miscellaneous. Among the five general lectures on the program of the Eighth International Congress of Applied Chemistry is one on "The rôle of very small amounts of chemical substances in biochemistry," by Gabriel Bertrand.

Dr. Simon Flexner delivered a lecture on "Infection and Recovery from Infection," in the auditorium of the U. S. National Museum on February 8. This was the third Hamilton fund lecture of the Smithsonian Institution.

At the third lecture in a series on biological topics, at Trinity College, Prof. Frederic S. Lee recently exhibited, for the first time in this country, cinematograph films representing phases of muscular action, such as the reaction of the perfused heart to various agents, ciliary and flagellate motions in *Trypanosoma*, etc. Professor Lee also recently lectured before the International Y. M. C. A. Training School at Springfield, Massachusetts, on "Some aspects of muscular action."

Prof. Francis E. Lloyd gave an account of his recent work on the tannin content of the acorn of *Quercus laurifolia* before the Botanical Seminar of Johns Hopkins University, on February 16, and a few days later before the Journal Club of the Department of Zoology, of Columbia University. A digest appeared in the Johns Hopkins University Circular for February, under the title, "The association of tannin with an emulsion colloid in the acorn."

Prof. J. J. R. Macleod will spend the rest of the year, until the opening of the next session, in Europe. During May and June he will deliver a series of eight lectures on "Carbohydrate metabolism" in the course of advanced lectures in physiology offered by the University of London.

Prof. Lafayette B. Mendel gave an illustrated lecture, before the Columbia chapter of Sigma Xi, January 18, on "Recent studies on nutrition and growth." Professor Mendel also lectured before the students of Mt. Holyoke College, on March 2, on "Changing views on nutrition."

Dr. Harvey W. Wiley delivered the annual oration on medicine at the recent annual meeting, in Albany, of the Medical Society of the State of New York.

Professor C.-E. A. Winslow delivered a lecture on sanitation and public health, on Mar. 5, at the University of Pittsburgh under the auspices of the department of sanitary engineering.

A series of seven lectures on "public health" subjects was delivered at the University of Illinois, on Wednesdays and Fridays, beginning March 13. The subjects and lecturers were "Sociological aspects of public health," by Dr. Henry B. Favill; "Influence of water supplies on public health," by Prof. Edward Bartow;

"How not to be sick," by Dr. John N. Hurty; "Bovine tuberculosis and its relation to public health," by Dr. Mazýk P. Ravenel; "The influence of disease on civilization," by Dr. Victor C. Vaughan; "Ventilation of schoolrooms and bedrooms," by Dr. William A. Evans; "Milk," also by Dr. Evans.

Buildings and general equipment. *Dispensary for Syracuse Medical College.* It is reported that the trustees of Syracuse University have decided to erect a teaching dispensary near the medical college, to cost about \$75,000.

Peter Bent Brigham Hospital. It is expected that the Peter Bent Brigham Hospital, now under construction on land adjoining the Harvard Medical School, will be completed in October, 1912. Dr. Henry A. Christian, retiring dean of Harvard Medical School, will be physician-in-chief; Dr. Harvey Cushing, of Johns Hopkins Medical School, surgeon-in-chief; and Dr. H. B. Howard, superintendent.

Graduate school of medicine at Harvard. About a year ago a graduate school of medicine was established as a department of Harvard University and placed under the control of the faculty of medicine. Recently Dr. Horace D. Arnold was elected Dean. The new feature of this school is not the giving of graduate instruction in medicine, since that has been encouraged by Harvard Medical School for a number of years. The action taken is a recognition that graduate instruction was important enough to justify the establishment of a separate department of the university and that this department should be developed along the same high plane as that of the other departments. With the rapid improvement that the undergraduate medical schools of this country have been experiencing, it is highly important that the graduate schools undergo an even greater development so that medical graduates will have opportunity for post-graduate instruction in medicine at least as thorough and as scientific as that furnished in the best undergraduate medical schools. Furthermore, it is probably a fact that graduate medical instruction in this country, generally speaking, has been even more neglected than undergraduate instruction, and there is much greater need of improvement. The creation of this new department at Harvard University, therefore, is a step in the right direction.

Harvard a medical center. Within a radius of half a mile of Harvard Medical School there are already built or under construction sixteen medical institutions, representing in value of buildings and capitalized funds about \$20,000,000. The five new hospitals, two of which will move into new quarters, added to the eleven medical institutions already constructed, will make the hospital and clinical facilities of the school extraordinary. The buildings at present under construction are the Harvard Memorial Cancer Hospital, the Thomas Morgan Rotch, Jr., Building (which will house the Infant's Hospital), the Children's Hospital, the Peter Bent Brigham Hospital, the State Psychopathic Hospital, the Robert Brigham Hospital, and a Harvard dormitory. A medical union club house will probably soon be added to the group.

Brooklyn botanic garden. Excavation was recently begun for the first section of the laboratory building and plant houses of the Brooklyn Botanic Garden. The building, when completed, will be one story high, of brick faced with concrete, 240 feet long and 50 feet wide, with a maximum elevation of about 60 feet.

Research in biology in the tropics. The University of Porto Rico announces that it plans to offer, to students, facilities for research in botany and zoology in the American tropics. Special space will be reserved in the agricultural building now in process of erection and the well-equipped physical, chemical, botanical, zoological, bacteriological and plant pathology laboratories may be drawn upon for supplies and apparatus. The research laboratories will be equipped only with the usual essentials, but endeavor will be made to meet the special needs, in the way of equipment, which the problems of each student demand. Free tables will be provided for a limited number of advanced students under conditions which will be explained upon communication with the director. Students who desire to use the laboratories are requested to communicate as early as possible, to the director, Dr. F. L. Stevens, their needs and dates at which accommodation is desired, since there is often delay in procuring supplies.

New York State School of Agriculture. Governor Dix has signed the Harte bill providing for the establishment of a New York State School of Agriculture on Long Island and appropriating \$50,000 for that purpose.

Scripps Institution for Biological Research. Control of the Marine Biological Station of San Diego has been transferred to the regents of the University of California. The official designation under the new regime will be the Scripps Institution for Biological Research of the University of California. Although, as indicated by the change of name, an extension of activities is contemplated, no immediate alteration of policy or work will take place. Dr. William E. Ritter will continue as the scientific director.

New buildings. The Massachusetts Institute of Technology has received, from a donor whose name for the present is anonymous, a gift of two and a half million dollars for the erection of the buildings on its new site.

Research laboratories in chemistry. Columbia University has recently received a gift of \$30,000 from Dr. William H. Nichols for instruction and research laboratories in chemistry.

Special funds. *Endowments.* The tenth yearbook of the Carnegie Institution of Washington is of special interest, as it records a further gift from the founder of \$10,000,000 and reviews the history of the institution for its first ten years. The endowment is now \$22,000,000 in five per cent. bonds of the steel corporation, worth at least \$25,000,000. The investment in property of the institution from its income is about \$1,700,000 and there is a reserve fund of \$250,000. During the ten years the cost of administration has been \$400,000, of publication \$300,000, and the sum of \$4,000,000 has been applied directly to research. There have been published 201 volumes under 156 different titles. The departments of biological sciences received last year grants amounting to \$90,000 and the Nutrition Laboratory had an appropriation of \$30,000.

The treasurer of Columbia University recently reported to the trustees that he had received about \$1,550,000 from the executors of the estate of the late George Crocker. Accordingly, the work of cancer research, for which Mr. Crocker gave this sum as an endowment, will begin at once. The research fund will be intrusted for administration to a board of managers, to consist of Mr. Rives, Dr. Cheesman and President Butler representing the trustees, and Dean Lambert, Professors Janeway and MacCallum, of the medical faculty, together with a director of cancer research to be appointed.

The million dollar fund for the further endowment of the Medical School of Western Reserve University has been completed.

The Illinois State Supreme Court has rendered a decision which declares unconstitutional an act of the last legislature which voted an item of \$60,000 for the medical school of the university.

The Committee on Scientific Research of the American Medical Association has charge of a small fund for the promotion of research. The committee desires that this money be used to meet actual needs and to promote investigative work not otherwise adequately provided for. Applications for grants are invited. The applicant should state fully the purpose for which the grant is desired, as well as the qualifications of the applicant, and the general opportunities and conditions under which the work would be done. The members of the committee are Drs. Ludvig Hektoen (1743 West Harrison Street, Chicago), Graham Lusk and Eugene L. Opie.

The widow of the late Professor Hitzig has given 84,000 marks to the Prussian Academy of Sciences for the encouragement of researches on the brain.

Mr. Albert Plaut has donated \$5,000 to Princeton University to be used as a fund for encouraging the study of chemistry at that university by securing distinguished chemists to address the Chemical Club.

Gifts. Lord Lister bequeathed nearly the whole of his fortune to scientific institutions and hospitals, including \$100,000 to the Lister Institute of Preventive Medicine and \$50,000 to the Royal Society.

Prof. Theodore C. Janeway recently presented to Columbia University the medical library of his father, Dr. Edward G. Janeway, to be the nucleus of the library of the Department of Practice of Medicine. The library comprises about 1500 bound volumes and 2,500 unbound monographs, journals and pamphlets. It was also announced that Mrs. Russell Sage has given \$25,000 to establish the E. G. Janeway library endowment fund at the Columbia Medical School.

Mr. John D. Rockefeller has contributed \$11,000 toward the purchase of the house in which Pasteur was born, in the village of Dôle.

Prizes. The Naples Table Association for Promoting Laboratory Research by Women announces a prize of \$1,000 for the best thesis written by a woman on a scientific subject, embodying new observations and new conclusions based on an independent laboratory research in biological (including psychological), chemical, or physical science. The theses offered in competition must be presented to the executive committee of the association and must be in the hands of the chairman of the committee on the prize, Dr. Lilian Welsh, Goucher College, Baltimore, Md., *before February 25, 1913*. The prize will be awarded at the annual meeting in April, 1913. Each thesis must be submitted under a pseudonym and must be accompanied by a sealed envelope, enclosing the author's name and address, and superscribed with a title corresponding to one borne by the manuscript. Additional information may be obtained from the Secretary, Ada Wing Mead (Mrs. A. D.), 283 Wayland Avenue, Providence, R. I.

There will be awarded in 1915 a prize of 300 francs to the author of the best memoir on the improvement of present anesthetic processes, or to the author of a discovery of an anesthetic considered worthy of the attention of the jury, or to the inventors of apparatus, processes or methods markedly facilitating anesthesia. The memoirs may be addressed to M. Quincerot, *président du Comité Horace Wells*, 28, rue de Moscou, Paris. The memoirs must be written in French. A device should accompany the manuscript, also a sealed envelope bearing on the outside the device, and containing the name and address, of the candidate.

Grant. The directors of the Bache Fund of the National Academy of Sciences have voted a grant of \$500 to Prof. M. A. Rosanoff, of Clark University, in aid of his research on the dynamics of sugar inversion.

Journalistic. *A Review of Internal Medicine.* The fact that the *Zentralblatt für innere Medizin*, which has been in existence for a number of years, has been for a long time insufficient for the requirements of scientific internists, led the members of the Congress of Internal Medicine to develop a plan to found a new organ which should appear under the auspices of the organized internists of Germany. The plan has been developed and the first number of

the organ will be published in a few weeks, under the title: *Zentralblatt für die gesamte innere Medizin und ihre Grenzgebiete*. The journal will be conducted by a committee appointed by the Congress (for the present consisting of von Müller of Munich, von Noorden of Vienna, and His and Schwalbe of Berlin), and, like the *Chemische Zentralblatt*, will consist exclusively of abstracts. These will cover as completely as possible the whole extent of internal medicine and its allied fields. In this way the new *Zentralblatt* will be the most comprehensive in the world's medical literature. The annual subscription will be about \$30 (120 marks). The members of the Congress for Internal Medicine receive a discount of 25 per cent. Furthermore, the old *Zentralblatt für innere Medizin* will be continued under the leadership of Dr. A. Schmidt of Halle, but it will be intended especially for internists who are not engaged in scientific work and for general practitioners.

New mycological journal. We have received a copy of the first (March) number of the *Zeitschrift für Gärungsphysiologie*. This new journal will be devoted to general, agricultural and technical mycology, under the editorship of Prof. Alexander Kossowicz, of Vienna (Wien II, Josef-Gall-Gasse 2). The subscription price is 20 Marks per volume of about 400 pages. Publishers: Gebrüder Borntraeger (Berlin W 35, Schöneberger Ufer 12 a).

Dr. Wiley takes up a new issue. The magazine *Good Housekeeping* announces that Dr. Harvey W. Wiley, formerly chief chemist of the United States Department of Agriculture, has become contributing editor of that magazine, which will hereafter be the exclusive channel for his writings on pure food, health and similar topics. The magazine has established, at Washington, a "Bureau of Foods, Sanitation and Health," of which Dr. Wiley will be in full charge and control as director.

Herter memorial journal. The title page of the *Journal of Biological Chemistry*, beginning with Vol. X, contains the memorial legend: "*Founded by Christian A. Herter and sustained in part by the Christian A. Herter memorial fund.*" (See page 140.)

Change of editors. Mr. Selskar M. Gunn, assistant professor in the department of biology and public health of the Massachusetts

Institute of Technology, has become the editor of the *Journal of the American Public Health Association*, succeeding Dr. Burt R. Rickards.

Dr. George B. Shattuck has retired from the active editorial management of the *Boston Medical and Surgical Journal* after a service of thirty-one years.

Miscellaneous notes. *Sodium benzoate: a correction.* The *Journal of the American Medical Association* has lately commented as follows (Jan. 20, p. 199) on its previous discussion of the expert opinion of the Royal Scientific Deputation for Medical Affairs regarding the use of benzoic acid and its salts for the preservation of food: "We were not justified in concluding that the criticism of the Royal Scientific Deputation for Medical Affairs referred to the whole of the work done by the Referee Board. The *Journal* has no desire to be unfair in any comments it may make on the benzoate of soda controversy and it regrets the error that was made in this particular. At the same time we believe that the attitude of the American Medical Association on the question of the use of sodium benzoate in food is the correct one, and we shall continue to protest against the use of this chemical as a food preservative. And we again call attention to the fact that while the Prussian scientists accepted, at their face value, the findings of the Referee Board, they reached the following conclusion in their own report: '*The Scientific Deputation for Medical Affairs is likewise of the opinion that the use of benzoic acid and benzoic acid salts for the preservation of food should not be permitted.*' And when all is said and done this, of course, is the nub of the whole question."

Sodium benzoate: a judicial decision. Some time ago the benzoated products of several manufacturers of prepared foods were barred from Indiana by the State Board of Health. The ground of objection was their content of benzoate or benzoic acid. The food manufacturers applied for a federal restraining order, on the plea that benzoate and benzoic acid in "small amounts" are harmless. The Master in Chancery, who heard the case, recently reported adversely to the manufacturers. Apparently his finding was based on the conviction that the manufacturers failed to show the

alleged harmlessness of "small amounts" of benzoate and benzoic acid.

Retiring allowances. The University of Chicago has established a system of retiring allowances for professors or their widows. A fund of \$2,500,000 taken from the \$10,000,000 Rockefeller gift of 1910 has been set aside for this purpose. This pension system will grant to men who have attained the rank of assistant professor or higher, and who have reached the age of 65 and have served 15 years or more in the institution, 40 per cent. of their salaries and an additional 2 per cent. for each year's service over 15. The plan also provides that at the age of 70 a man shall be retired unless the board of trustees specially continues his services. The widow of any professor entitled to the retiring allowance shall receive one half the amount due him, provided she has been his wife for ten years.

Rev. Stephen Hales, D.D. Francis Darwin has written, for the *Parish Magazine*, an interesting statement regarding Rev. Stephen Hales, D.D., one of the most distinguished men of science of the eighteenth century (1677-1761), from which we quote the following: "Stephen Hales has been called the '*father of physiology*' and he deserves this title in regard both to animals and plants. His experiments on the blood pressure of animals are second only to Harvey's work on the circulation. In the domain of plant physiology he is equally great. In all his researches he combined a belief in the design of the Creator with a passionate desire to understand the mechanism of living things. Thus he treated the manifestations of life as things to be weighed, measured and analyzed in the laboratory. It is this point of view that gives his work so modern a character and entitles him to be considered one of the founders of a rational science of biology. Although he loved science for its own sake, it is equally clear that he was dominated by a permanent desire to use his knowledge for the benefit of his fellow-creatures. Water supply, ventilation, the distillation of potable water at sea, the preservation of food on long voyages, the treatment of at least one disease—the stone—and especially the harm arising from intemperance in the use of alcohol, all received attention. It is im-

possible to read his works without mingling personal affection with the respect inspired by his intellect."

On the value of classical instruction. In an article on the value of classical instruction in the preparation of young people for the technical schools, M. Henry Le Chatelier, professor at the Sorbonne and member of the Academie des Sciences, emphasizes the importance of pure and clear style in scientific writings, and compares American and English scientific papers, saying that most of the latter are absolutely unreadable; all the American papers are clear and comprehensible, even monotonously so, as if written by the same pen. "I have more than once," declared M. Le Chatelier, "on receiving an unreadable thesis for the doctor's degree in chemistry, said to its author, 'Open the *Journal of the American Chemical Society*, select one of the papers in this publication and imitate the style of the American chemist in describing your own results.'"

Artificial production of larvæ of Lepidoptera. The experiments which were performed by Tichomorow on the effects of sulfuric acid in inducing the development of eggs of Lepidoptera have been recently repeated by Mr. L. B. Ripley, of Trinity College, with positive results. The eggs were taken from females which had been isolated since their emergence from the pupal cases and treated for five seconds with concentrated sulfuric acid, after which they were washed in water. Larvæ resulted which are now feeding upon wild cherry. Tichomorow did not succeed in obtaining living larvæ.

Therapeutic research. It is often said that therapeutics, and especially drug therapeutics, have not kept step with the progress of medical science. The reiteration of the fact may become somewhat tiresome, but it serves a useful purpose, in that it will gradually lead to a correction of the fault. An attempt in this direction is being made by the Council on Pharmacy and Chemistry, through the appointment of a Committee on Therapeutic Research, and the Board of Trustees of the American Medical Association has made an appropriation for this work. This is in direct line with the basic objects of the Council—namely, the advancement of therapeutics by substituting definite knowledge for vague impressions and general "beliefs." . . . The number of practical urgent problems in

therapeutics is so great, and their nature so varied and complex, that the most which the members of the Council could accomplish by personal research would be inadequate to improve the situation seriously. The Committee therefore judged that it would be most useful by supporting and facilitating such researches. . . . In practice, the Council aims to select the lines which appear most promising; to interest competent investigators to take charge of these lines; and to render to them such assistance as it can. . . . In the selection of problems, emphasis will be laid mainly on their feasibility and practical importance. *All who are interested are invited to submit suggestions along these lines.* . . . The Committee realizes that it has undertaken a very large task, the outcome of which depends on the coöperation which it can enlist, rather than on its personal endeavors. On the whole, the task is a hopeful one, provided that one does not demand immediate or startling returns. I shall not be disappointed so long as the Committee engages the active and continuous interest of even a single competent investigator. . . . Suggestions, applications and other communications to the Committee should be addressed to its Secretary, Prof. W. A. Puckner, 535 Dearborn Ave., Chicago. (*Sollman: Journal of the American Medical Association*, 1912, lviii, p. 1390.)

Practical monopoly of the sale of radium. An official report announces that the Austrian government has bought up all the mines of Joachimsthal (Bohemia), where radium is present. Extensive investigations by experts have shown that it will be possible to obtain per year from these mines at least a ton of uranium ore, equivalent to 3 grams of radium, valued at \$240,000. Together with the production of the other state mines, the yearly output of this precious substance will amount to about 5 grams. Austria will thus practically monopolize the sale of radium, for the mines in California, in Spain and in Saxony cannot compete with the Austrian. The bulk of the year's output will be handed over to the Radium Institute in Vienna, which will use it for extensive experiments. A large quantity will be disposed of to hospitals, and the surgical clinics in Vienna will soon have the largest quantities possessed by any charitable institution in the world. Small quantities will be sold to physicians and scientists. The mines were

bought for nearly \$500,000. The cost of "manufacturing" 1 gram of radium is very low (only \$4,000), while the by-products are very valuable, for nearly 6,000 pounds of uranium colors are obtained in the process of separating 1 gram of radium. This uranium is valued at \$20,000. Thus the government has made a very good bargain, and it may easily place large quantities of the precious material at the disposal of clinical teachers for the benefit of the diseased poor. (See page 348.)

American Medicine gold medal. Editorial attention is given in a current issue of *American Medicine* (p. 188) to the subject: "Scientific research in the United States receives too little recognition." A sympathetic discussion of this matter is followed by editorial announcement that "the editors of *American Medicine* have established the American Medicine Gold Medal for Conspicuous Contributions to the Progress of Medical Science. It is to be donated each year to the American surgeon, physician or investigator who, in the opinion of three trustees to be announced in our (their) May issue, has made the most noteworthy contribution to medical science."

Eighth International Congress of Applied Chemistry: German committee of biological chemists. Professors Gottlieb of Heidelberg, Heffter of Berlin, Kossel of Heidelberg and Thierfelder of Tübingen, are coöperating with the American officers of the Section (8, d) of Physiological Chemistry and Pharmacology in furthering the interests of biological chemistry and biological chemists at the Eighth International Congress of Applied Chemistry to be held in Washington and New York, September 4-13, 1912.

Book review: *Principles of Human Nutrition. A study in practical dietetics.* By Whitman H. Jordan. Cloth, xxi + 450 pp., index. \$1.75 net. The Macmillan Company, New York.

Professor Springer, of Minnesota, has aptly expressed the idea that as a scientific subject comes within the sphere of common knowledge and contributes directly to the comfort and well being of the race, it becomes necessary for that topic to be moved downward in the curriculum of our educational system. Facts and principles discovered in the laboratory and applied in a practical

manner soon become the common property of the laity, but are usually imperfectly understood and will render their best service only when people are properly instructed in regard to them.

The rapid advances in the medical sciences in the last two decades have had so many practical applications to daily life that many facts and principles of nutrition are already becoming matters of common knowledge, and it is very important that these should be properly understood by the public so that every one can avail himself of the best means of sound living, and guard against mischievous errors.

Dr. Jordan's recently published book is an attempt to set forth in simple terms the fundamentals of human nutrition, and, as he himself says, "to connect them with a philosophy of living," so that the student without technical training in chemistry and biology can learn his own food requirements and how to meet them, both of which are equally important.

The author, though an expert in animal nutrition, has the layman's point of view and presents the normal rather than the pathological aspects of his subject, a fact which makes the book of wider interest than many others dealing with foods and feeding. The subject matter is excellently presented and in the main correct in detail. The author fails to recognize that the formation of sugar from protein has been definitely established and unfortunately underestimates the value of such ash constituents as iron. On the practical side the author warns against food fads. He advocates a simple and well-balanced dietary, and presents a number of suggestive illustrations.

The book is timely, in that courses in this subject are now being introduced extensively in agricultural schools, high schools, and private schools throughout the country. Dr. Jordan's book acceptably meets the new needs in this connection and is the best of its kind that has yet appeared.

A. R. R.

II. PHARMACOLOGICAL LABORATORY, BUREAU OF CHEMISTRY,
U. S. DEPARTMENT OF AGRICULTURE

The pharmacological laboratory of the Bureau of Chemistry was organized by order of the Secretary of Agriculture on June 22, 1908, for the purpose of testing the action of drugs and drug products. At first it occupied quarters in a private house near the Bureau of Chemistry, but in February, 1910, the Bureau of Chemistry moved to its new quarters, where the pharmacological laboratory comprises a room for chemical work, an operating room for physiological work, two rooms for animals, and an office for the chief.

The laboratory is well equipped at present for the study of the action of drugs and other substances, and their effects on man and animals. Besides much routine testing (which is not published) the workers in this laboratory have conducted investigations on bleached flour and on the effects of caffeine, the various alcohols and other compounds.

The pharmacological laboratory was organized under the personal direction of Dr. William Salant, who has been its chief from the beginning. The following papers have been presented from this laboratory by Dr. Salant and his associates.

1908. William Salant: The necessity for animal experimentation in determining the purity and strength of medicinal preparations; *Proceedings of the Twenty-fifth Annual Convention of the Association of Official Agricultural Chemists, Bul. 122, Bureau of Chemistry, U. S. Department of Agriculture*.

1909. William Salant: The comparative toxicity of ethyl and amyl alcohol and their effects on blood pressure; *Proceedings of the Society for Experimental Biology and Medicine*, vi, p. 134.—W. O. Emery and William Salant: On the decomposition of caffeine in the liver; *Ibid.*, vi, p. 132.

1910. William Salant: The toxicity of amyl acetate; *Ibid.*, vii, p. 154.—William Salant and W. O. Emery: The elimination of caffeine in the bile; *Ibid.*, vii, p. 155.—William Salant and J. B. Rieger: Caffeine tolerance; *Journal of Pharmacology and Experimental Therapeutics*, i, p. 572 (Proc. Amer. Soc. Pharm. Exp.

Therap.).—William Salant and J. B. Rieger: The toxicity of caffeine; *Ibid.*, i, p. 572.—William Salant and G. W. Knight: Observations on caffeine glycosuria: *Proceedings of the American Society of Biological Chemists*, i, p. 265.

1911. William Salant: The pharmacology of oil of chenopodium; *Journal of Pharmacology and Experimental Therapeutics*, ii, p. 391 (Proc. Amer. Soc. Pharm. Exp. Therap.).—William Salant and J. B. Rieger: The elimination of creatin and creatinin after the administration of caffeine; *Ibid.*, ii, p. 401.—William Salant and I. K. Phelps: The influence of caffeine on protein metabolism in dogs, with some remarks on demethylation in the body; *Ibid.*, ii, p. 401.—William Salant and J. B. Rieger: The influence of alcohol on protein metabolism in dogs; *Proceedings of the American Society of Biological Chemists*, ii, p. 6.—William Salant: The action of drugs under pathological conditions; *Circular No. 81, Bureau of Chemistry, U. S. Department of Agriculture*. Pp. 16.

1912. William Salant: The effect of caffeine on the circulation; *Journal of Pharmacology and Experimental Therapeutics*, iii, p. 468 (Proc. Amer. Soc. Pharm. Exp. Therap.).—William Salant and I. K. Phelps: Demethylation of caffeine and theobromin under pathological conditions; *Ibid.*, iii, p. 469.—William Salant and J. B. Rieger: The elimination of caffeine; *Ibid.*, iii, p. 469.—William Salant and J. B. Rieger: The toxicity of caffeine; *Ibid.*, iii, p. 455.—William Salant and J. B. Rieger: The toxicity of caffeine; *Bulletin No. 148, Bureau of Chemistry, U. S. Department of Agriculture*. Pp. 98.—William Salant and J. B. Rieger: The elimination and toxicity of caffeine in nephrectomized rabbits; *Proceedings of the Society for Experimental Biology and Medicine*, ix, p. 58.—William Salant and J. B. Rieger: The elimination of caffeine; *Bulletin No. 157, Bureau of Chemistry, U. S. Department of Agriculture*. Pp. 23.—William Salant: Further studies on the toxicity of caffeine. (*In preparation*—a continuation of Bulletin 148.)

III. COLUMBIA BIOCHEMICAL ASSOCIATION¹

Change in the Secretaryship. Dr. Walter H. Eddy, who has served with exceptional efficiency as secretary of the Association

¹ Notes pertaining to members appear among the other groups of items (pp. 492-518).

since its establishment two years ago, has resigned. The increasing demand upon Dr. Eddy's time in other important relations has induced this decision, very greatly to the regret of all the members. Prof. Wm. H. Welker has been elected to succeed Dr. Eddy until the next annual meeting of the Association in June.

Proceedings of the Association. *Fourth scientific meeting*² (First annual lecture). The fourth scientific meeting of the Association, a largely attended public session, was held in Rumford Hall of the Chemists' Club, on the evening of March 15. Prof. Alexander Smith kindly served as honorary President of the meeting. On this occasion Prof. Wilder D. Bancroft was the guest of the Association and delivered a very interesting and instructive lecture on the subject of "Environment." An abstract of the lecture is published at page 382 of this issue of the BULLETIN. The lecture was followed by an animated discussion, in which the chief participants were Professor Bancroft, Drs. L. H. Baekeland, Jacques Loeb, S. J. Meltzer, C. Stuart Gager and A. J. Goldfarb, Professors Graham Lusk and Carlton C. Curtis, and Messrs. B. C. Gruenberg and Carl A. Schwarze. At the close of the discussion Professor Gies formally expressed the Association's indebtedness to Professor Bancroft for the instruction it had received from his lecture and for the pleasure it had derived from his visit.

About forty members and guests enjoyed an informal dinner at the Chemists' Club before the lecture.

Lecture on fasting. Dr. Paul E. Howe, Instructor in Physiological Chemistry at the University of Illinois, will deliver a public lecture, under the auspices of the Association, at the College of Physicians and Surgeons, May 1 at 11 a.m., on the subject of *Fasting*. Dr. Howe has made extended studies of nutritional problems and will discuss *fasting* from the standpoint of a special investigator of the subject.

Newly elected officers and members of various societies. Recent elections of members of the Association to office, and to membership, in scientific societies are indicated in the appended summary (or in the lists on pages 493-5).

² *First* scientific meeting, Feb. 28, 1911 (page 62); *second* (second annual meeting), June 5, 1911 (page 332); *third* (first annual dinner), Dec. 13, 1911 (page 334).

Officers. Prof. P. B. Hawk: Chairman of the University of Illinois section of the American Chemical Society.

Dr. Alwin W. Pappenheimer: Secretary of the New York Pathological Society.

Dr. A. D. Selby: Councillor of the American Phytopathological Society.

Dr. William A. Taltavall: Treasurer of the San Bernardino County (Cal.) Medical Society.

Mr. Harry L. Fisher: *Councillor* of the national organization of Phi Lambda Upsilon; Mr. Harold E. Woodward, *Vice-President*, and Mr. Edward G. Griffin, *Treasurer* of the Columbia (Gamma) Chapter of Phi Lambda Upsilon. (Page 354.)

Dr. Ralph G. Stillman, *President*, Dr. Clinton B. Knapp, *Member of the Executive Committee* and Dr. Wm. K. Terriberry, *Member of the Nominating Committee*, of the Nu Sigma Nu Alumni Association.

Members. Drs. Louis Baumann and Isidor Greenwald: Members of the American Society of Biological Chemists.

Dr. Alfred E. Cohn: Member of the American Physiological Society.

Dr. C. A. Darling: Member of the Columbia Chapter of Sigma Xi.

Drs. George Draper and C. C. Lieb: Members of the Society for Experimental Biology and Medicine.

Dr. Max Morse: Member of the American Chemical Society.

Dr. A. Franklin Shull: Member of the American Society of Zoologists (Central Branch).

Journalistic. Dr. Geo. D. Beal is the Editor of the *Phi Lambda Upsilon Register*.

Miss Jean Broadhurst has been reelected an associate editor of the *Bulletin of the Torrey Botanical Club*.

Dr. Fred J. Seaver is the Editor of the *Journal of the New York Botanical Garden* and an associate editor of *Mycologia*.

Appointments. Prof. Russell Burton-Opitz: Member of the executive faculty of the College of Physicians and Surgeons.

Dr. Frederic M. Hanes: Assistant professor of pathology at Columbia University.

Dr. Louis D. Mead: Assistant in medicine; assigned to the department of pediatrics of the medical school of Leland Stanford, Jr., University.

Dr. A. Franklin Shull will be one of the instructors at the University of Michigan's station for instruction and research at Bogardus Engineering Camp, Michigan, during the fourth summer session, July 2–August 23, inclusive.

Dr. Louis E. Wise, lately chief analyst and research chemist with Schieffelin and Company, has become research chemist at the Standard Varnish Works, Staten Island, New York.

Miscellaneous. Dr. William N. Berg has begun his new work in the Pathological Division of the Bureau of Animal Industry, of the U. S. Department of Agriculture.

Dr. Louis J. Curtman recently published a very complete set of "*Tables pertaining to soluble inorganic compounds for the use of chemists, pharmacists and teachers of analytical and general chemistry.*" Biological chemists will find the tables very serviceable.

At the celebration of the one hundredth anniversary of the founding of the Academy of Natural Sciences of Philadelphia, held March 18–21, Dr. C. Stuart Gager represented the Torrey Botanical Club and also the University of Missouri.

A chemical laboratory of the most modern construction has been built for the Carnegie Station for Experimental Evolution at Cold Spring Harbor, Long Island. This building will be equipped with all the apparatus and material necessary for a biochemical study of the problems of heredity in plants and animals. Dr. Ross A. Gortner will direct the biochemical phases of this work.

Dr. R. F. Hare is head of the Department of Chemistry of the New Mexico College of Agriculture and Mechanic Arts, and Agricultural Experiment Station. He and his staff are busily engaged at present on a chemical study of New Mexico soils and waters in coöperation with the U. S. Geological Survey.

Dr. H. D. House has presented to the New York Botanical Garden a collection of 163 specimens of fleshy and woody fungi

secured by him in the forests of Germany during the autumn of 1911.

An extremely handy volume by Dr. A. D. Selby on plant diseases, consisting of a general treatment, a special part on Ohio plant diseases, and a classified bibliography, has recently been issued as Bulletin 214 of the Ohio Agricultural Experiment Station.

IV. COLUMBIA BIOCHEMICAL DEPARTMENT

Professor Gies has recently delivered addresses before a Lutheran Brotherhood in Jersey City, on "Life and some of its mysteries" (Jan. 16); before the New York Institute of Stomatology, on "The need for chemical investigation of dental caries" (Feb. 19); the Columbia chapter of Sigma Xi, on "The chemistry of digestion" (Mar. 13); and the Pennsylvania chapter of Sigma Xi, on "Chemistry in the service of biology" (Mar. 29). He represented the Gettysburg Board of Trustees among the speakers at the dinner of the New York Gettysburg Club in honor of his Yale classmate, President Granville of Gettysburg College (April 10). He will also address the Franklin Institute, Philadelphia, on "Chemical defences of the organism against disease" (Apr. 25), and the Forty-fourth Annual Meeting of the Dental Society of the State of New York, on the "Origin, significance and possible relation of sulfocyanate to dental caries" (May 10).

Dr. Mosenthal is now in Europe devoting himself to a continuance of his studies of nephritis, under the auspices of the Edward N. Gibbs Memorial Prize Fund.

At the request of about sixty students at Teachers College, Professor Gies is giving a special course of weekly lectures there on "Nutrition in disease."

Dr. Rosenbloom recently delivered a lecture, by invitation, before the Pittsburgh Academy of Medicine, on "The chemistry of diabetes mellitus" (Apr. 9).

Dr. Clark has been reëlected an associate editor of the *Bulletin of the Torrey Botanical Club*.

Dr. Eddy has been elected a member of the American Chemical Society.

Professor Gies has been complimented by numerous newspaper nominations for the position in the Bureau of Chemistry made vacant by Dr. Wiley's resignation (pages 391 and 523) and by hearty endorsements for it from many colleagues. To us in the department, Professor Gies promptly made it evident that he is not a candidate for the position and has no desire to leave Columbia.

Messrs. Smith and Rose, of the staff, and M. L. Hamlin, John L. Kantor, C. A. Mathewson, E. C. Stone and Harold E. Woodward, advanced students in the department, were recently elected to membership in the Columbia chapter of Sigma Xi.

Mr. Smith was lately elected a member of the Columbia (Gamma) chapter of Phi Lambda Upsilon. Professor Gies was one of the speakers at the chapter's annual dinner (April 1).

Dr. Max Kahn, who is about to complete the requirements for the Ph.D. degree, has been appointed director of the biochemical and clinical laboratories of the Beth Israel Hospital, New York City.

Courses in nutrition (physiological and pathological chemistry) will be conducted in the biochemical laboratories at the College of Physicians and Surgeons and Teachers College during the summer session by Professor Gies with the coöperation of Drs. Emily C. Seaman and Clayton S. Smith. The laboratory will be open all summer for research.

EDITORIALS

From an early period of his career Lister was deeply impressed by the great mortality of operations and severe injuries attended by external wounds, such as compound fractures. In those days

Joseph Lister almost every operation was followed by what was termed "surgical fever." Lister became acquainted with the great discovery of Pasteur, that fermentation and putrefaction are due to microbes. He recognized that the wound complications were due to putrefaction from the same cause and grasped the surgical possibilities. His first idea was to kill the germs already admitted to wounds and then to prevent the entrance of others. The first agent he selected for this purpose was phenol (carbolic acid). His articles, which inaugurated the antiseptic system, were published in 1867 in the *Lancet* under the title: "A New Method of Treating Compound Fractures, Abscesses, etc." He freely applied phenol to the interior of the wounds in order to destroy the air-borne germs, and covered the opening skin with lint charged with phenol and protected by an external layer of thin sheet metal. His results showed a marvelous improvement. Hospital gangrene, pyemia and erysipelas, which had been frequent in his wards, disappeared. With indefatigable industry he constantly experimented to improve his technic, using in turn phenolized putty, phenolized shellac, the phenol spray, perchlorid of mercury, sal alembroth and double cyanid of mercury and zinc.

Lister's appointment as professor of clinical surgery at King's College gave greater opportunities for the diffusion of his teaching, which now was receiving much attention abroad, as well as at home. Indeed, the antiseptic system was earlier recognized by foreign than by British surgeons. The conservatism of his own country proved a strong barrier, as it has always done, to reforms, however great and beneficent. Some of the leading surgeons of the day not only refused to adopt the antiseptic system, but violently opposed it. Even a man of the eminence of Sir James Simpson

wrote a lengthy article to prove—what was already admitted—that Lister was not the first to use phenol and that his methods were not original. Sir William Savoury, the most eloquent surgeon of the day, who enjoyed the unprecedented distinction of being president of the Royal College of Surgeons for five years in succession, threw ridicule on the antiseptic system. Lawson Tait, the greatest and most original gynecologist of the day, adopted the same attitude. But, like Darwin, Lister never descended to controversy; he was too busy with research. So bitter was some of the opposition to him in high places that, incredible as it may seem, the Royal College of Surgeons never honored itself (for that is the way to put it) by appointing him president. It was said of him in 1900 that he had saved more lives than had been destroyed in all the wars of the century. Proposing his health at a dinner of the Royal Society, Mr. Bayard, the American ambassador, said, "My lord, it is not a profession, it is not a nation, it is humanity itself which salutes you."

Lister made one discovery which, even apart from the antiseptic system, would entitle him to enduring fame—the absorbable catgut ligature. He found that silk ligatures were a source of trouble in his treatment of wounds because of the irritation and suppuration which sometimes followed. He experimented by tying the arteries of dogs and calves with catgut and found that it underwent complete absorption without causing suppuration. He applied the result to man and was able to announce that surgeons "may now tie an arterial trunk in its continuity close to a large branch, secured alike against secondary hemorrhage and deep-seated suppuration." He subsequently evolved the chromicized catgut ligature. He made many other important contributions to surgical knowledge which have been overshadowed by his great discovery.

As a man, Lister was singularly modest and unassuming, and his solicitude and gentleness with patients were almost feminine. It is related of him that when speaking to the great Austrian surgeon, Billroth, who was skeptical about the antiseptic system, he simply said, "If you tried it I am sure you would be pleased with it." [*Journal of the American Medical Association*, lviii, p. 645; March 2.]

"I flatter myself that you may read with some interest what I have written on the organisms which you were the first to describe in your works. I do not know whether the records of British surgery ever meet your eye. If so, you will have seen, from time to time, notices of the antiseptic system of treatment, which I have been laboring at for the last nine years to bring to perfection. Allow me to take this opportunity to tender you my most cordial thanks for having by your brilliant researches, demonstrated to me the truth of the germ theory of putrefaction and thus furnished me with the principle upon which alone the antiseptic system can be carried out." *Lister to Pasteur.*

On the occasion of the great celebration in honor of Pasteur in 1892, there occurred a memorable incident which, we are told, softened the hearts of all: As Lister finished his congratulatory address on behalf of the Royal Societies of London and Edinburgh, Pasteur rose and embraced him, and, writes Pasteur's biographer, "the sight of these two men gave the impression of a brotherhood of science laboring to diminish the sorrows of humanity." [*Journal of the American Medical Association*, 1912, lviii, p. 486; Feb. 17.]

His brow spreads large and placid, and his eye
Is deep and bright, with steady looks that still.
Soft lines of tranquil thought his face fulfil—
His face at once benign and proud and shy.
If envy scout, if ignorance deny,
His faultless patience, his unyielding will,
Beautiful gentleness and splendid skill,
Innumerable gratitudes reply.
His wise, rare smile is sweet with certainties,
And seems in all his patients to compel
Such love and faith as failure cannot quell.
We hold him for another Herakles,
Battling with custom, prejudice, disease,
As once the son of Zeus with Death and Hell. *Henley.*

It is incorrect to speak of modern surgical practice as opposed to Lister's teaching. "Aseptic surgery is a direct development of

antiseptic surgery: it represents simply a new and improved way of applying the same principles." Lister saw clearly what he was about. "We may dispense entirely with irrigation," he said, "whether in the form of the spray, which was a kind of irrigation, or in any other; in fact, our operations may be performed with just the same simplicity as in former years. What we have to attend to is to prevent the entrance into our wounds, during operations, of the grosser forms of septic mischief, such, for instance, as exist in impure sponges or dirty instruments, or in any unclean material upon our hands or on the skin of the patient." It was a question of means. New and better means than the wholesale application of carbolic acid were ultimately devised, but the end in view is still the same.¹

The portrait of Lister, at the front of this issue (page 371), is presented by courtesy of *American Medicine*, having first appeared in the March issue of that valuable monthly. We are greatly indebted to *American Medicine* for permission to reproduce the portrait and for the use of the plate for that purpose.

Waldemar Koch was highly esteemed by all who knew him.² His personal friends were legion, his professional admirers were a multitude. Halliburton said, several years ago, that Koch was one of the few biological chemists who had had the courage to undertake the study of brain chemistry.³ Koch's researches in recent years were rapidly carrying him toward the solution of some of the most perplexing problems in neurochemistry and the many results of his work in this field (page 374) will exercise a continuing constructive influence on the future investigations of the chemistry of the nervous system. A month before his death, Koch was elected Secretary of the Section of Physiology and Experimental Medicine (K) of the American Asso-

¹ "In the natural process of evolution, asepsis later succeeded antiseptis, and regarding this matter Championnière, the father of asepsis, stated that "aseptic surgery is as much an issue of the discovery of Lister as is true antiseptic surgery." *Journal of the American Medical Association*, 1912, lviii, p. 499.

² See page 372.

³ Halliburton: Oliver-Sharpey Lectures, *British Medical Journal*, 1907 (May 4).

ciation for the Advancement of Science, and was appointed Chairman of a committee of the American Biochemical Society to consider and report on the nomenclature and classification of the fats and fat-like substances. One of his last scientific statements was a formal expression of his conviction that protagon, by whatever process it may be made, is a *mechanical mixture* of at least three substances and that "the term protagon cannot therefore be said to have any chemical significance."¹

Dr. Wiley's resignation has been received with almost universal regret.² The public has had unlimited confidence in Dr. Wiley, personally as well as in his capacity effectively to serve it profession-

The resignation of Dr. Wiley ally. The people believe that Dr. Wiley's official acts were above any suspicion of self-interest or of unfaithfulness to duty. His success has occasioned very general gratification.³

A number of biological chemists have derided Dr. Wiley's professional ability, and have openly expressed doubt of his sincerity and integrity. Eminent men of science have contended that Dr. Wiley was unfit, as a man and as a chemist, to direct the great work of the Bureau of which he was chief. The temperamental qualities which have helped to carry Dr. Wiley very far in public approval have stamped him as a charlatan in the minds of some who have evidently tried to understand him and to correctly estimate his work.⁴

The public has sized up Dr. Wiley through a telescope, so to speak,—and has seen the best of him! Some "experts," on the other hand, have sized him down through a microscope and, in aiming to get a "better definition," appear to have suffered severely from eye strain.

It is impossible for the writer (not an expert) to believe that the general public estimate of Dr. Wiley is incorrect. It has been conclusively demonstrated that during his long term of service, Dr.

¹ Koch: Proceedings of the American Society of Biological Chemists (December, 1911), 1912, ii, p. 74; Journal of Biological Chemistry, 1912, xi, p. x1.

² See page 391.

³ See page 394 for a statement of prevailing medical opinion of Dr. Wiley and his work.

⁴ Lusk: Medical Record, 1911, lxxx, p. 1184 (Dec. 9).

Wiley made a number of mistakes. That he did not know everything in chemistry or biology was cheerfully confessed very often by Dr. Wiley himself. He neither claimed nor pretended to be an up-to-date biological chemist, but his practical knowledge of agricultural chemistry places him among the distinguished scientists in that particular field. His keen comprehension of the dangers to the health of the people from the use of impure food and drugs, and his success in advancing measures to prevent the distribution of fraudulent nutritional and medicinal products, give him high rank as a public sanitarian. It was vastly more important and serviceable for Dr. Wiley, as chief of the Bureau of Chemistry, to be an expert in these matters, than to know the constitutional formula of acetanilid. If he was "crooked" or a "grafter," as some "experts" whisper, it seems singular that no one has come forward openly with facts to prove him dishonest or degraded.

Dr. Wiley's official conduct appeared to be characterized by the policy: "When in doubt give the public the benefit of the doubt." That such an administrative policy cannot always be wholly right or ultra-scientific is obvious, but it accords with the plain duty of official public service, and is certainly better and safer for the greatest number of people involved than the opposite policy, or the rule: "Never be in doubt—be cock sure!"

The writer believes that Dr. Wiley's administration of the Bureau of Chemistry will long be held in grateful remembrance for the earnest, able and successful performance of duty which characterized it. Its defects—and every administration has many of them—will be recalled in the light of a generous estimate on the personality of a strong, zealous, faithful and genial public servant. We hope Dr. Wiley will enjoy many more years of usefulness in the great work to which he has been consistently devoted.

I. O. N.

Attention may be called to one or two helpful publications on the biochemistry of plants or animals. For some reason the excellent "*Pflanzenchemie*" of Hans Euler, professor of chemistry in

the University of Stockholm, does not seem to be known to many who would be interested in a modern concise presentation of the physiology of plants. The work

is in two small volumes, very well organized from the printer's standpoint and the material is clearly and definitely put.

The *first volume* deals with the organic chemistry of plants. The subject receives different treatment from that of many of the texts in organic chemistry purported to aid especially the biochemical student, and which begin with the simple hydrocarbons (few of which appear at all in the economy of organisms, plant or animal) and work thence to the alcohols, aldehydes, etc. Euler's book begins with the alcohols and only the compounds of the nitrogen-free aliphatics, the nitrogen-free cyclic and the nitrogenous compounds (alkaloids, amins, purins, amino acids, polypeptids and proteins) are considered which have any interest to the student of the chemistry of living things. Brief qualitative and quantitative data are included after the discussions of the compounds; and their distribution in plants and likewise in animals is mentioned. The *second volume* is confined to the general laws of plant life, with special reference to the physical chemistry of the various processes. The second half of it is devoted to a discussion of the chemical processes. While there is, therefore, a distinction in regard to these two phases, there is no unnatural limitation tending to classify organic processes into those concerned with the chemistry of the carbon atom and the phenomena of surface tension, osmosis, etc.

The second publication, also concerned with the biochemistry of plants, is a small primer by Frederick Czapek the well-known author of the two-volume "*Biochemie der Pflanzen*." The little volume is entitled "*Chemical Phenomena in Life*" and is one of Harper's Library of Living Thought. The point of view is principally that of the physical chemist undertaking a rationale of plant processes, protoplasm being considered as proteins in the colloidal state, the cell-membrane being considered in the light of permeability and semi-permeability, enzymes being considered in the light of the inorganic catalyzer data and the laws of mass action, etc. It is an excellent book for the teacher to put into the hands of the beginner in biochemistry, for if this does not stimulate an interest in the subject, it is doubtful whether anything else will.

The excellent treatment of the physico-chemical phenomena of the animal body which Hedin (Upsala) contributed to Mandel's

latest English presentation of Olof Hammarsten's Text-book has been referred to elsewhere. Aside from personal differences of opinion in regard to a few things, it seems that the treatment is such as to appeal to all students and investigators. If such workers will not go to original sources and to such finished products as those of Höber, Freundlich and others, published in foreign tongues, they may well find a resumé in Hedin which has a modern touch.

A digest of certain phases of the subjects referred to above is made by MacClendon in the *Biological Bulletin* for February, 1912 (22: 113). The article is suggestive of certain lines of research which may profitably be carried out.

Mention may be made of the General-Register of the *Biochemische Zeitschrift* (Volumes 1-30) which places the wealth of this journal in a form available to the reader. At the same time, it is a favorable commentary on the growth of the science and speaks ably against the views held by some who are unable to appreciate biochemistry as a science.

M. M.

In the Propaganda for Reform Department of this issue are given the results of an investigation carried on in the Association's laboratory to determine whether or not **Not a square deal for the physician** there is any chemical difference between acetphenetidin and phenacetin. As every physician knows, phenacetin was for some years the proprietary name of the substance known, chemically, as acetphenetidin. When the patent on phenacetin expired, the substance became public property. Nevertheless, the original patentees, the Farbenfabriken of Elberfeld Company, still list and sell the product under its proprietary name and also offer it under its chemical name. *Under the proprietary name, the company demands more than five times the price it asks for the same product sold under its chemical name. The same anomaly is perpetuated in the United States by the only American firm that makes the product under both its proprietary and its chemical names—Lehn and Fink.* Other American pharmaceutical houses sell the substance only under its official name. The investigations of the Association's chemists show that the only

demonstrable difference between phenacetin and acetphenetidin is the price. *Verbum sat sapienti!* [*Journal of the American Medical Association*, 1912, lviii, p. 787; March 16.]

Four years ago, when the constitution of the American Society of Biological Chemists was adopted, the following provision was ratified by a large majority:

Professional code of ethics Any member who may grant the use of his name for (a) the advertisement of a patent medicine, a proprietary food preparation, or any other commercial article of doubtful value to the public or possibly harmful to the public health, or (b) who may concede its use for the purpose of encouraging the sale of individual samples (of any such product) that he has not examined, shall forfeit his membership.¹

Members of the Biochemical Society have lately suggested that this provision be eliminated from the Society's constitution. We commend to their notice and to the attention of all who think as they do, the following "Report of the Committee of the American Chemical Society, Industrial Division, on Professional Code of Ethics":²

Your Committee beg to report that after a careful consideration of the subject it is their belief that a code of ethics should be formulated and adopted by the American Chemical Society.

There are many instances which can be cited of unethical practices among chemists, such as: misleading advertisements and interviews in papers and magazines; the lending of certificates for advertising uses; the misuse of our science for the purpose of perpetrating frauds on the public, such as the evasion of a standard of purity or the sophistication of a product; slovenly and unreliable work sometimes revealed by impossibly low prices for analytical tests; expert testimony of a character discreditable to the witness and to our profes-

¹ Constitution of the American Society of Biological Chemists, Article III, Membership; Section 4, Forfeiture—A: Proceedings of the American Society of Biological Chemists, 1908, i, p. 85.

² Presented at the forty-fifth meeting of the American Chemical Society, Washington, December, 1911. Reprinted from the *Journal of Industrial and Engineering Chemistry*, 1912, iv, p. 226.

sion; a lack of courtesy towards our fellow chemists: and in this respect we stand far below the medical profession.

The present committee of three should be retired, and a larger committee, of perhaps seven members, be appointed, representing the various branches of our profession, and with power to draw up and recommend a code of ethics. Such work should be done deliberately and with the greatest possible discussion, which can not fail to have a beneficial effect, in pointing out evil practices and elevating our standards of behavior. This subject has already been discussed at Indianapolis, Louisville and New York, and we believe that each of the several sections would do well to devote one night to the discussion of this problem.

We do not think that for the present, at least, it will be possible or desirable for the American Chemical Society to attempt the enforcement of a code of ethics, still less to examine members with a view to certifying their competency as is done by the Institute of Chemistry of Great Britain; but we do feel that the adoption of a carefully worked out code of ethics, prominently displayed in our publication, would set before us the standard of professional conduct, which, as members of the American Chemical Society, we would be expected to follow, and which would greatly tend to emphasize our brotherly relations to fellow chemists and elevate the ideals of our profession.

(Signed) A. C. LANGMUIR, C. F. MCKENNA, L. F. BROWN,
Committee.

We noted recently, with great surprise, that the new edition of the Encyclopedia Brittanica fails to give special
Cholesterol attention to cholesterol, although such substances as chlorpicrin receive more than adequate notice.

I know of no surer index of a man's greatness than the measure of inspiration imparted by him.—*Martin*.

A really great man is known by three signs—generosity in
Ions design, humanity in execution and moderation in success.—*Bismarck*.

An earnest endeavor to separate scientific truth from the influence of psychic turmoil must be the aim of the scientific man.—*Lusk*.

The humanity which would prevent human suffering is a deeper and truer humanity than the humanity which would save pain or death to animals.—*Eliot*.

OFFICERS OF THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY. 1911-1912

WILLIAM J. GIES, M.S., Ph.D., <i>Professor</i> . Chairman of the Staff.	ERNEST D. CLARK, A.M., Ph.D., <i>Instructor</i> .
WILLIAM H. WELKER, A.C., Ph.D., <i>Assistant Professor</i> .	REUBEN OTTENBERG, M.D., <i>Assistant</i> .
NELLIS B. FOSTER, M.D., <i>Associate</i> .	ANTON R. ROSE, A.M., <i>Assistant</i> .
WALTER H. EDDY, A.M., Ph.D., <i>Associate</i> . Secretary of the Staff.	CLAYTON S. SMITH, B.S., <i>Assistant</i> .
JACOB ROSENBLOOM, M.D., Ph.D., <i>Associate</i> .	EDGAR G. MILLER, JR., A.B., <i>Assistant</i> .
ALFRED P. LOTHROP, A.M., Ph.D., <i>Instructor</i> . Department Registrar.	CHRISTIAN SEIFERT, <i>Laboratory Ass't</i> .
HERMAN O. MOSENTHAL, M.D., <i>Instructor</i> .	STELLA WALDECK, <i>Recorder</i> .
EMILY C. SEAMAN, A.M., <i>Instructor</i> .	CONSTANCE C. HART, <i>Laboratory Assistant</i> .
	BLANCHE R. HARRIS, <i>Laboratory Assistant</i> .
	M. V. MILLER, A.B., <i>Laboratory Assistant</i> .

COURSES OFFERED BY THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY. 1911-1912

Courses 51 and 105 are given during the first half year only. Course 101 is given during the first half year and is repeated (102) during the second half year. Courses 104 and 106 are given only during the second half year. All other courses are conducted throughout the entire academic year. All courses not otherwise specified are given at the College of Physicians and Surgeons.

(*Abbreviations:* C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51. ELEMENTARY ORGANIC CHEMISTRY. Introductory to courses 101, 102 and 104. (*Required of first year students of medicine.*) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, and Mr. Smith.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101-102. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (Teachers College, School of Household Arts.) L, 1 hr. R, 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Miss Seaman and Mr. Miller. (This course is designated "H. A. 25a" in the Teachers College Announcement.)

This course is designated "S—H. A. 25" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies and Miss Seaman.

104. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R, 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, Dr. Clark, and Messrs. Smith and Rose.

This course is designated "S—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Mr. Smith.

106. GENERAL PATHOLOGICAL CHEMISTRY. *Lectures on nutrition in disease.* (Teachers College, School of Household Arts.) L, 1 hr. Prof. Gies. (This course, given this year for the first time, is designated "H. A. 25b" at Teachers College.)

201-202. CHEMISTRY OF NUTRITION. (School of Pharmacy. *Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

203-204. GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* L, 1 hr. Lw, 4 hr. Dr. Eddy.

Courses in Nutrition (continued)

205-206. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (Teachers College, School of Household Arts.) L, 1 hr. Lw, 5 hr. Prof. Gies and Miss Seaman. (This course is designated "H. A. 125" in the Teachers College Announcement.)

207-208. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS. L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Lothrop.

209-210. NUTRITION IN HEALTH AND DISEASE. L, 2 hr. Prof. Gies.

211-212. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies, Mr. Rose, and Dr. Clark.

213-214. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies.

215-216. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Welker, Drs. Lothrop and Clark and Mr. Rose.

TOXICOLOGY

217-218. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. Lw, 6 hr. Prof. Gies.

BOTANY

219-220. CHEMICAL PHYSIOLOGY OF PLANTS. (New York Botanical Garden.) L, 1 hr. Lw, 5 hr. Prof. Gies and Dr. Clark.

BACTERIOLOGY

221-222. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Clark.

SANITATION

105. SANITARY CHEMISTRY. (Teachers College, School of Household Arts.) L, 1 hr. Lw, 3 hr. Professor Gies, Miss Seaman and Dr. Clark. (This course is designated "H. A. 26, a" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301-302. BIOCHEMICAL SEMINAR. 1 hr. Prof. Gies.

RESEARCH IN BIOLOGICAL CHEMISTRY

Biochemical research may be conducted, by advanced workers, independently or under guidance. Prof. Gies consults regularly with investigators in the laboratories of Botany and Zoology on Tuesday afternoons, and at the N. Y. Botanical Garden on Wednesday afternoons.

BIOCHEMICAL LIBRARY

Prof. Gies' library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons and is accessible, by appointment, to all workers in the Department.

LABORATORIES FOR ADVANCED WORK IN BIOLOGICAL CHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College and the New York Botanical Garden. The laboratories are well equipped for research in nutrition and all other phases of biological chemistry.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

The Biochemical Association holds quarterly scientific meetings, which are open to all students in the University.

SUMMER SCHOOL COURSES

Summer session courses are mentioned in the foregoing references to Courses 101-102 and 104. Prof. Gies will have charge of both courses next summer. He will also conduct a special lecture course in nutrition. The laboratories will be open for research during the summer session.

Biochemical Bulletin

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THE BIOCHEMICAL BULLETIN

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The BIOCHEMICAL BULLETIN will promptly acknowledge, under this heading, the receipt of all publications that may be presented to it. From time to time, selections will be made for review on pages of the volume to be appropriately indicated here. Reviews will be matter-of-fact statements of the nature and contents of the publications under consideration, and will be 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 mechanistic conception of life. *Biological essays.* By Jacques Loeb, Member of Rock. Inst. for Med. Research. Pp. 232; \$1.65. Univ. of Chicago Press, Chicago, 1912.

Digestion and metabolism. *The physiological and pathological chemistry of nutrition.* By Alonzo Englebert Taylor, Rush prof. of physiol. chem., Univ. of Penn. Pp. 560; \$3.75 net. Lea and Febiger, Phila., 1912.

Monograph on biochemistry. *Soil conditions and plant growth.* By Edw. J. Russell, Goldsmiths' soil chemist, Rothamstead Exp. Sta'n, Harpenden, Eng. Pp. 168; \$1.50 net. Longmans, Green and Co., N. Y., 1912.

Monograph on biochemistry. *The physiology of protein metabolism.* By E. P. Cathcart, Grieve lect. on chem. physiol., Univ. of Glasgow; Research Associate, Carnegie Inst. Pp. 142; \$1.25 net. Longmans, Green and Co., N. Y., 1912.

Ueber elektrostatische Zellkräfte und mikroskopischen Elektrizitätsnachweis. By Rudolf Keller, Prag. Pp. 142; unbound: 4.20 Marks. J. G. Calve, k. u. k. Hof- und Univ.-Buchhändler, Robert Lerche, Kleiner Ring 12, Prag, 1912.

The colloidal and crystalloidal state of matter. By Paul Rohland, A. O. Prof., Techn. Hochsch., Stuttgart; trans. by W. J. Britland and H. E. Potts. Pp. 54; \$1.25 net. D. Van Nostrand Co., N. Y., 1911.

A text book of medical chemistry and toxicology. New ed. (3d), revised. By James W. Holland, prof. of med. chem. and toxicol., and Dean, Jeff. Med. Col. Pp. 655; \$3.00 net. W. B. Saunders Co., Phila., 1911.

Practical medical chemistry. By Charles Platt (London) and Wm. A. Pearson (Phila.). New ed. (6th). Pp. 260; \$2.50 net. John Jos. McVey, Phila., 1911.

Triumphs and wonders of modern chemistry. *A popular treatise on modern chemistry and its marvels, written in non-technical language for general readers and students.* By Geoffrey Martin, lect. on chem., Birkbeck Coll., London Univ. Pp. 358; \$2.00 net. D. Van Nostrand Co., N. Y., 1911.

An essay on hasheesh. *Includes observations and experiments.* By Victor Robinson, contrib. ed., Med. Review of Rev. Pp. 83; \$0.50 net. Med. Rev. of Reviews, N. Y., 1912.

BIOCHEMICAL BULLETIN

VOLUME I

JUNE, 1912

No. 4

A STUDY OF ROPY BREAD

ANNA W. WILLIAMS

(Research Laboratory, Department of Household Science, University of Illinois, Urbana, Ill.)

Introduction. Ropiness in bread has recently been receiving considerable attention from investigators. However, its causes and more especially the methods for its prevention are not yet thoroughly understood by the average baker.

Various spore-forming bacilli belonging to the potato group have been proved to cause ropiness, but *B. mesentericus vulgatus* (Flügge) seems to have been most often identified. Lafar¹ made note of Orth's separation of three potato bacilli from slimy bread, one of which gave a red color and was found to be harmless, while the other two were considered as the cause of the disease. Watkins² obtained a pink colored crumb in a bread made from rope-infected flour, but did not report any specific bacillus as the cause.

The source of infection in most cases has been found to be the flour, although occasionally the yeast has been held to be responsible. All investigators agree that moisture and warmth are favorable for the development of the bacilli and that, if bread made from infected flour be kept at a sufficiently low temperature, ropiness will not develop. Watkins has shown that acidity, produced by the presence of lactic or acetic acid in the dough, retards and, when present in sufficient amounts, entirely inhibits the growth of the organism.

The writer's attention was drawn to this study because of the

¹ Lafar: Handbuch der technischen Mykologie, 1908, ii, p. 522.

² Watkins: Ropiness in flour and bread, and its detection and prevention; Jour. Soc. Chem. Indus., 1906, xxv, p. 351-354.

constant appearance of infection in the bread of the immediate vicinity. In one family attempts had been made to discover the cause of the trouble, but without success. The yeast had been varied, the bread had been most thoroughly baked, and the flour had been changed, but the odor and flavor peculiar to ropy bread continued to develop within a few days after baking. However, the same flour bin and utensils had been used persistently, and the bread had been kept in a tight bread-box.

Investigations were begun in order (1) to discover the cause of the infection, and (2) to determine some methods of prevention practical for the housewife. With these purposes in view the following experiments were carried out, the ordinary short process method of bread-making being used.

Experiment 1. The aim of this experiment was to determine the cause and source of the infection.

Method. A flour considered as sound was first proved to contain no rope-producing bacilli. This was done by making a loaf of bread and incubating it at the same degree of moisture and temperature as a corresponding loaf to which some infected flour had been added. The fact that under exactly the same conditions the latter loaf developed ropiness while the former did not was taken as evidence that the former flour was sound. This sound flour was then used in all control work, and when inoculations were made it was used as the medium.

Portions of the suspected flour were tested by mixing it with sound flour and making breads. These breads were incubated at various temperatures and under various conditions of moisture. The suspected flour was also mixed with water and boiled for 20 minutes, after which it was used to inoculate sterile bread sticks according to Watkins' method.

Hanging drop studies were next made from some infected bread. Gelatin-plate, gelatin-stab, and agar-streak cultures were made from the same material. Capsule stains were subsequently tried. Tables 1 and 2 show the results of this experiment.

A study of the hanging drop showed a considerable number of short, sluggishly-motile rod-shaped bacilli, and some cocci. On staining, the bacillus showed the presence of a capsule. Inoculated bread sticks developed ropiness.

TABLE 1.

Data indicating the cause and source of the ropy infection

No.	Conditions of baking		Incubation			
	Nature of flour used	Conditions		Results		
		Moisture	Temperature	44 hrs.	6 days	11 days.
1 (Control)	Sound flour.	Kept moist with sterile water.	40°	—	—	Mold, but no signs of rope.
2	Sound flour + 1 tsp. infected flour.	Kept moist with sterile water.	40°	Faint odor of rope.	Distinct brown patches.	Crumb completely brown and ropy.
3	Sound flour + 1 tsp. infected flour.	Kept dry.	40°	—	—	Very faint odor of rope.
4	Sound flour + 1 tsp. infected flour.	Kept slightly moist.	20°	—	—	Very faint odor of rope.
5	Sound flour + 1 tsp. infected flour.	Kept very slightly moist.	Icebox	—	—	No signs of ropiness.
6	Sound flour + 3 tsp. infected flour.	Baking not thorough. Kept moist with sterile water.	40°	Brown spots visible. Distinct ropy odor.	Crumb almost entirely brown	

TABLE 2.

Additional data pertaining to the cause and source of the ropy infection.

Culture	Results
Gelatin plate.	Transparent, ropy growth, turning medium yellowish-green, and causing complete liquefaction.
Gelatin stab.	Growth practically invisible, liquefaction napiform, taking place in 10 days at room temperature.
Agar streak.	Transparent growth.

Discussion of the results. The hanging drop study, accompanied by the cultural characteristics, show the cause of the rope to be a short, sluggishly motile, rod-bacillus, apparently the *B. mesentericus vulgatus* (Flügge) obtained by previous investigators.^{1, 2}

The results in Table 1 and in the inoculated bread-sticks show that the bacillus is found in the flour. The results also show that both warmth and moisture are necessary for its growth, and that

¹Watkins: Ropiness in flour and bread, and its detection and prevention; Jour. Soc. Chem. Indus., 1906, xxv, pp. 353-354.

²Lafar: Handbuch der technischen Mykologie, 1908, ii, pp. 521, 522.

an increase in the concentration of bacilli in the flour, together with underbaking, increase rapidity of development. It is also seen that even when bread is made from infected flour, it is possible to prevent the growth of rope by dryness and low temperature after baking.

Experiment 2. The aim of this experiment was to devise a method of prevention that would be of practical use for the housewife.

Procedure. Flour was thoroughly inoculated by the transference of large portions of rope-infected bread to the flour. The mixture was subsequently incubated in a moist chamber at 50° C. for 48 hours. At the end of this time a portion of the flour was dried for 48 hours at 50° C.; a portion was sunned in a thin layer for 48 hours; and the remainder was made into breads, with hop-water and with different proportions of buttermilk. The acidity of the buttermilk used was 0.7 per cent. Table 3 shows the results of the baking experiments.

Discussion of the results. Within the limits of this experiment, sunning and drying of flour had no effect upon the bacillus that produced rope; neither did hop-water seem to be effective.

The results obtained by the use of different concentrations of bacilli in the flour, and of butter-milk, showed that when the entire loaf was made of the very strongly infected flour, even the exclusive use of butter-milk of 0.7 per cent. acidity as the liquid for mixing, failed to inhibit, or even retard noticeably, the development of rope. When a very small portion of infected flour was present in the bread, however, development of rope was slower; and the exclusive use of butter-milk, as the liquid for mixing, inhibited all growth of the rope; smaller quantities of butter-milk were, however, not effective. It seems, from these results, that the use of butter-milk in bread may be an effective preventive of rope, but the amount of butter-milk must be in proportion to the degree of infection of the flour. A flour may be so strongly infected, however, that the exclusive use of butter-milk will not prevent the growth of rope.

The breads in this experiment were all of excellent texture and of good flavor. A comparison of Nos. 6 and 7 shows that breads made from soft doughs, hence containing more moisture, develop

TABLE 3.
Results of the baking experiments.

No.	Ingredients of the dough		Conditions	Incubation			Results	
	Liquid	Condition of flour		16 hrs.	24 hrs.	48 hrs.		
1	Water.	All infected flour.	Moistened daily with sterile water; 40° C. incubation.	Brown patches.		Completely brown.		5 days
2	Water.	All infected flour; sunned for 48 hrs.	Moistened daily with sterile water; 40° C. incubation.	Brown patches.		Completely brown.		
3	Water.	Infected flour; dried at 59° C. for 48 hrs.	Moistened daily with sterile water; 40° C. incubation.	Brown patches.		Completely brown.		
4	Hop-water.	All infected flour.	Moistened daily with sterile water; 40° C. incubation.	Brown patches.		Completely brown.		
5	All butter-milk.	All infected flour.	Moistened daily with sterile water; 40° C. incubation.	Brown patches.		Completely brown.		
6	Water.	Sound flour + 1 tsp. infected flour. Slack dough.	Moistened daily with sterile water; 40° C. incubation.	Strong odor of rope.	Brown patches.		Completely brown.	
7	Water.	Sound flour + 1 tsp. infected flour. Stiff dough.	Moistened daily with sterile water; 40° C. incubation.		Odor of rope; br'n patches.		Completely brown.	
8	All butter-milk.	Sound flour + 1 tsp. infected flour.	Moistened daily with sterile water; 40° C. incubation.		Odor of rope.	Brown patches.	No signs of rope.	
9	B'termilk ($\frac{1}{2}$); water ($\frac{1}{2}$).	Sound flour + 1 tsp. infected flour.	Moistened daily with sterile water; 40° C. incubation.		Odor of rope.	Brown patches.	Completely brown.	
10	B'termilk ($\frac{1}{2}$); water ($\frac{1}{2}$).	Sound flour + 1 tsp. infected flour.	Moistened daily with sterile water; 40° C. incubation.		Odor of rope.	Brown patches.	Completely brown.	
11	Hop-water.	Sound flour + 1 tsp. infected flour; dried.	Moist'd occasion'ly with sterile water; 32° C. incubation.		Odor of rope.	Odor of rope.	Pink on cut surfaces.	
12	Water.	Sound flour + 1 tsp. infected flour; dried.	Moist'd occasion'ly with sterile water; 32° C. incubation.			Odor of rope.	Pink on cut surfaces.	
13	Butter-milk.	Sound flour + 1 tsp. infected flour; dried.	Moist'd occasion'ly with sterile water; 32° C. incubation.			Odor of rope.	Pink on cut surfaces.	

ropiness more rapidly than do those made from very stiff doughs. Nos. 11, 12, and 13 were slow in developing ropiness because the degree of infection was small, the temperature of incubation rather low, and the moisture not excessive. Ropiness was here, as in the other breads, first indicated by the odor. The usual ropy condition did not, however, develop, but the crumb became pink in the infected bread. No rods were apparent. Watkins³ makes note of an occasional development of a pink crumb accompanied by a ropy smell. Lafar⁴ mentions a bacillus occurring in ropy bread which gave a red color and was found to be harmless.

Conclusions. From the above results the following conclusions were drawn:

1. Ropiness in bread is produced by a rather short, thick, sluggishly motile, rod-bacillus possessing a capsule; this bacillus is found in some flours. Such rope-infected flour may also contain a coccus capable of producing a pink crumb on and near the cut surfaces of the bread; a characteristic odor of rope precedes the appearance of color. Such a development does not ordinarily take place, because it is slow, and ropiness develops first.

2. Bread made from infected flour may be prevented from developing rope by keeping the bread dry and at a low temperature.

3. Increased concentration of the bacilli in the flour and underbaking produce an increased rapidity in development of ropiness.

4. The stiffer the dough the more gradual the development of ropiness.

5. Butter-milk, if used as the liquid for mixing dough, will, in some cases, prevent the development of ropiness. The amount of butter-milk used must, however, be in proportion to the degree of infection of the flour. A flour may be so strongly infected that the exclusive use of butter-milk, of 0.7 per cent. acidity, will not prevent the growth of rope.

³ Watkins: Jour. Soc. Chem. Indus., 1906, xxv, pp. 351-354.

⁴ Lafar: Handbuch der technischen Mykologie, 1908, ii, p. 522.

THE PHYSICO-CHEMICAL BASIS OF STRIATED-MUSCLE CONTRACTION (I)

WILLIAM N. BERG

THE ZUNTZ THEORY OF MUSCLE CONTRACTION

In 1908 Zuntz¹ advanced the following theory of muscle contraction: Muscle fibrils consist of rods or cylinders having a diameter of 1 micron and a height of 6 microns. Assuming that but one half of the area is taken up by rods and the other half by sarkoplasm, a cross section of 1 c.c. of muscle would contain 62 million rods. Assuming that but one half the length of the fibril is occupied by the rods, there will be room, in 1 c.c. of muscle, for 800 layers, each containing 62 million rods. Zuntz calculates that these rods have an area of 8928 sq. cm. available for osmotic work.

The beginning of the contractile process lies in the combustion which takes place within the rods. The resultant carbon dioxid dissolves in the water present (as if it were so much sugar) and exerts an osmotic pressure of approximately 5 grams per sq. cm. At the moment of their formation, the carbon dioxid molecules have a temperature of over 6000° C. The osmotic pressure of the contents of the rods is raised, by this high temperature, to 462 grams per sq. cm.

As the result of this osmotic difference between the contents of the rod and the sarkoplasm bathing it, water diffuses rapidly into the rod, causing it to shorten and approach the spherical shape, *i. e.*, the muscle contracts. Presently the temperature falls through radiation, etc.; the osmotic pressure of the carbon dioxid falls; water diffuses outward, followed by a slower diffusion of the carbon dioxid and other products of muscular activity; *i. e.*, the muscle relaxes.

¹Zuntz, N.: Die Kraftleistung des Tierkörpers. Eine Festrede zur Feier des Geburtstages Sr. Majestät des Kaisers. Kgl. Landw. Hochschule, Berlin. 1908, Pp. 34.

THE AUTHOR'S CRITICISM OF THE ZUNTZ THEORY

The theory contains many objectionable features. Some of them are considered in the following brief review.

In his conception of the structure of human or frog muscle, Zuntz assumes that the fibril consists of a string of muscle rods. These rods exist in the living fibrils of certain insect muscles. The writer has not yet found any statement to the effect that they have been seen in frog or human muscle fibrils. Their existence in such fibrils is somewhat of an assumption.

His calculations contain the assumption that all of the oxygen consumed by a working muscle is used for the maintenance of combustion inside the rods, leaving no oxygen for the sarkoplasm, which constitutes the larger part of the muscle. Furthermore, it is not certain that when a molecule of fatty acid, for example, is burned in a working muscle, the entire combustion process takes place within the rod. It is possible that only a part of the entire heat of combustion is liberated in the rods, and there transformed into external work.

Practically all of the resultant carbon dioxid is present in the (sarkoplasm) lymph in a more or less strong combination with some lymph constituent. Only a very small portion of the total amount of carbon dioxid is present as gas in solution. Gases such as carbon dioxid and oxygen, when dissolved in water, do not behave entirely like true solutes. Until the osmotic pressure of a gas in solution has been measured, the statement that carbon dioxid exerts an osmotic pressure when dissolved in water should be regarded as one involving assumptions. This, of course, is not true of such gases as hydrochloric acid, ammonia, and a few others.

In view of the fact that carbon dioxid, at about 2000° C. is dissociated into carbon monoxid and oxygen, it is doubtful whether the carbon dioxid molecules at the moment of their formation within the muscle rod, have a temperature of 6000° C., as calculated by Zuntz. If they did, there would be carbon dioxid dissolved, not in water, but in steam (?) and under such conditions perhaps osmotic phenomena would not come into play at all.

It was not shown that the walls of the muscle rods are impermeable to carbon dioxid during the contraction phase. This is

necessary, for otherwise, osmotic equilibrium could be brought about, not alone by an inflow of water, but by an outflow of carbon dioxid as well. According to the theory, there is an outflow of carbon dioxid during the relaxation phase, implying a change in the permeability of the rod walls. Insofar as this change in permeability is only one of a series of changes that together constitute muscular contraction and relaxation (which, in certain cases, can take place in a very few hundredths of a second), it is obviously desirable to establish the fact that the rod-wall has a permeability (to carbon dioxid, for example) that can be varied over wide limits in a very short space of time.

Even granting that the osmotic pressure inside the rod is 462 grams per sq. cm. greater after combustion than it was before, the movement of water from the sarkoplasm into the rod ought not to be thought of as if it were going from a region of zero to a region of 462 grams per sq.cm. osmotic pressure, as Zuntz evidently does. The osmotic pressure of the constituents of the lymph bathing the rods is approximately 6000 grams per sq. cm. and Zuntz's calculated increase, 462 grams per sq. cm., is but 8 per cent. of this amount. Zuntz calculates that the amount of water entering the rods, as the result of the combustion, may be over 50 per cent. of the volume of the rods.

According to Hürthle, who measured the lengths and diameters of the rods in the muscle fibrils of certain insects, the volume of the rods remains unchanged during contraction and relaxation.

Washington, D. C.

A FURTHER STUDY OF THE BARDACH TEST FOR PROTEIN

CHARLES WEISMAN

(Laboratory of Biological Chemistry of Columbia University, at the College
of Physicians and Surgeons, New York)

Bardach's protein test,¹ shortly after its publication, was subjected to a general examination in this laboratory. It was found that every protein, of many that were selected for trial, promptly yielded the reaction,² thus strengthening the general conclusion which Bardach drew from a few observations.

Dr. Gies recently concluded that Bardach's test might be given by various simple cleavage products of proteins and that possibly the responsiveness of proteins in the test is due to radicals that also appear in amino acids, hexone bases, or related substances. At his suggestion I have made an inquiry into this matter, with the results described below.

Bardach's test depends upon the fact that (in the presence of protein) acetone and iodo-potassium iodid and alkali react to yield canary yellow *needles* of an unidentified nitro material, instead of the usual *hexagonal* crystals of iodoform. The test may be conducted as follows: To 5 c.c. of the solution under examination (concentration of protein should not exceed 5 per cent.), add 2-3 drops of 0.5 per cent. solution of acetone, then sufficient Lugol solution³ to supply a moderate excess of iodine and to produce a reddish-brown color; and finally an excess of ammonium hydroxide (about 3 c.c. of concentrated solution). The required amount of Lugol solution depends on the quantities of protein, sugar and other iodine-reacting substances that may be present, and may vary from one drop to several cubic centimeters. The precipitate forms within a short time, usually within an hour, and if it consists of canary yellow needles, protein is indicated. The crystals tend to form rosettes, bundles, or knobbed and branching variations.

¹ Bardach: Zeitschrift für physiologische Chemie, 1908, liv, p. 355.

² Seaman and Gies: Proceedings of the Society for Experimental Biology and Medicine, 1908, v, p. 125.

³ Containing 4 grams of iodine and 6 grams of potassium iodid per 100 c.c. of water.

If a moderate excess of iodine is used in the test, and protein is present, a black precipitate of iodo-nitro products is usually formed after the addition of ammonium hydroxide, upon which, after its sedimentation, yellow crystals are deposited. If just the proper amount of iodine is present the liquid soon becomes yellowish and any black precipitate that may have been formed upon the addition of ammonium hydroxide is gradually transformed more or less completely into the yellow needles. In the presence of too great an excess of iodine the reaction may be obscured or even prevented. Insufficient iodine or too much protein may also prevent the reaction. When excesses of both protein and iodine are present, a grayish-green precipitate may form on adding ammonium hydroxide. Under such conditions, if the proportions are favorable and the mixture is stirred for a few moments, the precipitate is gradually transformed into the yellow needles.

In this extension of the work of Seaman and Gies, we determined the behavior of the following substances in aqueous solutions:⁴ alanine, asparagine, aspartic acid, neutral arginine nitrate, glycoll, glycyl-tryptophan, guanidine carbonate, hippuric acid, histidine dichloride, leucine, meta-phenylene diamine, toluene diamine hydrochloride, 1, 2, 4. Needles were formed with arginine nitrate, glycyl-tryptophan, and meta-phenylene diamine. Leucine yielded needles together with rosettes, the latter form predominating, while toluene diamine hydrochloride, 1, 2, 4, gave hairs, mainly in tufts.

Mixed products of protein hydrolysis were also studied. Tendon-collagen, elastin, commercial dry egg-albumen and ovo-mucoid were hydrolyzed with strong hydrochloric acid until the liquids no longer gave a biuret reaction. On then applying the Bardach test to the solutions of mixed hydrolytic products, negative results were obtained in each case except for ovo-mucoid. Characteristic needles appeared in the tests on the hydrolytic products of the latter substance. It is possible that arginin and leucin in the other solutions failed to yield needles either because of interference by associated products or insufficient concentration.

From the fact, as shown by the foregoing results, that simple amino substances yield a positive Bardach reaction, it is evident that this test, like a number of other "protein" tests, is not a specific one.

⁴ These products were free from biuret-reacting material.

STUDIES OF ENZYMES AS POSSIBLE FACTORS IN THE DEVELOPMENT OF EDEMA

4. The influence of proteases on the swelling of collagen and fibrin particles in alkaline and acid media containing a biological electrolyte

FRANK R. ELDER AND WILLIAM J. GIES

*(Laboratory of Biological Chemistry of Columbia University, at the College of
Physicians and Surgeons, New York)*

The chemical and physical coordinations between water and colloids in living protoplasm are among the most influential and important nutritional relationships.

Tracy and Gies recently observed that pepsin increases the absorption of water by particles of fibrin and collagen immersed in dilute solutions of hydrochloric acid, and that trypsin augments the hydrophilia of elastin particles in solutions of ammonium hydroxid and sodium carbonate.¹ Besides showing that both basic and acidic substances may help proteases to increase the absorption of water by protein colloids, the observations of Tracy and Gies suggest that hydrolases also appreciably influence the absorption of water by protoplasm. From this point of view, edema might be inaugurated by protoplasmic conditions that discoordinate normal enzymic relationships.

In the light of the foregoing facts and suggestions, the influence of neutral salins on enzymic stimulation of colloidal hydrophilia becomes a matter of special biological interest. It is well known that common neutral salin substances inhibit and, if sufficiently concentrated, entirely prevent the normal absorption of water by protein and other colloidal masses immersed in alkaline or acid media. Possibly the salins in and about living cells are also able to prevent special *enzymic* influences on water absorption and retention by protoplasm. The electrolytes in living protoplasm are conceivably

¹ Tracy and Gies: *Biochemical Bulletin*, 1912, i, p. 467. See also Berg and Gies: *Journal of Biological Chemistry*, 1906-7, ii, p. 489.

competent to inhibit completely the inauguration of edema by hydrolases.

We have made a preliminary inquiry into this matter by determining the influence of pepsin and trypsin on the imbibition of water by collagen and fibrin particles in suitable solutions containing sodium chlorid. Our study has been similar in method to the work described by Tracy and Gies.

Accurately weighed portions of dry protein were quantitatively transferred to tall, narrow, glass-stoppered, calibrated bottles, containing solutions of appropriate concentrations and exactly equal volumes. Enzyme solutions of precise preparation were then added in sharply measured amounts to the respective mixtures. Suitable control mixtures were made for each experiment. After their preparation, each mixture was gently shaken several times at intervals of about an hour. This treatment was always made mechanically uniform for each series of tests. Sedimentation was practically complete in an hour. The readings of bulk in c.c. on the calibrated scale were accurate within 1 c.c.

At the end of the period of observation, the liquids were separated from the protein masses. In the experiments with collagen, where digestive solution was slight, this was done by ordinary filtration, which was continued over night (12-24 hr.) for the complete removal of the free fluid. In most of the experiments with fibrin, digestive solution gradually became conspicuous; the liquids were accordingly strained quickly through pieces of muslin of identical size and the residual fluids completely expressed at once.

Each experiment was begun early in the afternoon and the observations were continued for from 22 to 46 hours, or longer. Environmental conditions were the same for each member of a series of tests at every stage of an experiment.

The data in Table 1 and 2 are typical of a group of about twenty experiments. The prevailing concentrations of hydrochloric acid and sodium carbonate, although not so favorable to our inquiry as certain others would have been, were arbitrarily selected to represent influences more nearly comparable with biological conditions than greater strengths would have afforded. The indicated concentrations of enzymes and salt were chosen, after preliminary tests, in order to develop maximal results under the conditions of cumula-

TABLE I

First series. Data showing the influence of pepsin on the absorption and retention of water by collagen particles in dilute hydrochloric acid solution containing sodium chlorid.

GENERAL DATA. The total volume of liquid in each test was 250 c.c., and the concentration of HCl was invariably 0.025%. Each mixture, except those of experiment 7, contained 2 grams of collagen. Experiments 1-3 were conducted with one preparation of collagen; experiments 4-7 with another. The pepsin product was the same throughout ("Pepsin, Merck: Powder—U. S. P., 01826").

Experiment 1. The strength of the stock solution of pepsin was 0.017% (0.1 gram of product in 600 c.c. of water); the proportion of pepsin product in the presence of the collagen was 0.000,041%.

Content of enzyme and saline		Bulk of the sedimented collagen mass (c.c.) at intervals during the progress of the experiment						Filtrate c.c.	Amount of water absorbed		
Pepsin solution c.c.	NaCl per cent.	Hours							Total c.c.	Excess over control	
		2	4	6	15	18	20			Absolute c.c.	Ratio to collagen, per cent.
0.6	0	201	206	208	209	213	213	122	128	3	150
0	0	198	203	206	208	210	210	125	125		
0.6	0.45	104	115	120	122	135	135	179	71	6	300
0	0.45	99	109	112	112	117	117	185	65		
0.6	0.90	66	82	90	91	102	100	198	52	2	100
0	0.90	64	70	84	84	88	88	200	50		

Experiment 2. The strength of the stock solution of pepsin was 0.067% (0.1 gram of product in 150 c.c. of water); the proportion of pepsin product in the presence of the collagen was 0.000,08%.

0.3	0	196	203	204	207	210	213	119	131	7	350
0	0	195	202	203	201	211	210	126	124		
0.3	0.45	94	107	111	114	129	132	182	68	3	150
0	0.45	108	116	117	118	123	123	185	65		
0.3	0.90	74	89	93	96	110	111	194	56	4	200
0	0.90	75	84	86	87	88	83	198	52		

Experiment 3. The strength of the stock solution of pepsin was 0.133% (0.1 gram of product in 75 c.c. of water); the proportion of pepsin product in the presence of the collagen was 0.000,16%.

0.3	0	198	204	205	208	219	219	116	134	16	800
0	0	195	201	204	204	208	210	132	118		
0.3	0.45	113	124	128	132	152	149	172	78	12	600
0	0.45	107	112	113	112	116	112	184	66		
0.3	0.90	84	94	97	101	117	119	192	58	8	400
0	0.90	77	84	85	85	88	88	200	50		

Experiment 4. In this and the remaining experiments of the series a new and more sensitive preparation of collagen was employed. The strength of the stock solution of pepsin was 0.25% (0.1 gram of product in 40 c.c. of water); the proportion of pepsin product in the presence of the collagen was 0.000,3%.

0.3	0.45	149	156	164	169	190	194	149	101	32	1600
0	0.45	138	143	145	146	150	150	181	69		
0.3	0.90	109	119	125	132	155	160	176	74	18	900
0	0.90	101	106	109	109	113	112	194	56		
0.3	1.20	88	97	103	109	132	135	191	59	12	600
0	1.20	81	86	88	90	93	92	203	47		

TABLE I (continued)

Experiment 5. The strength of the stock solution of pepsin was 0.5% (0.1 gram of product in 20 c.c. of water); the proportion of pepsin product in the presence of the collagen was 0.000,6%.

Content of enzyme and saline		Bulk of the sedimented collagen mass (c.c.) at intervals during the progress of the experiment						Filtrate c.c.	Amount of water absorbed		
Pepsin solution c.c.	NaCl per cent.	Hours							Total c.c.	Excess over control	
		2	4	6	15	18	20			Absolute c.c.	Ratio to collagen, per cent.
0.3	0.90	105	120	129	140	167	171	164	86	31	1550
0	0.90	98	104	108	110	113	113	195	55		
0.3	1.20	80	95	103	114	138	142	179	71	25	1250
0	1.20	78	86	89	90	91	92	204	46		
0.3	1.80	41	47	51	56	67	70	217	33	7	350
0	1.80	39	42	44	45	45	45	224	26		

Experiment 6. The strength of the stock solution of pepsin was 1% (0.1 gram of product in 10 c.c. of water); the proportion of pepsin product in the presence of the collagen was 0.001,2%.

0.3	1.20	79	99	108	128	156	164	164	86	41	2050
0	1.20	72	84	87	88	89	90	205	45		
0.3	1.80	39	47	51	62	76	78	211	39	14	700
0	1.80	39	43	45	45	45	46	225	25		
0.3	2.50	26	25	26	25	24	24	234	16	— 2	
0	2.50	26	25	26	26	26	26	232	18		

Experiment 7. The strength of the stock solution of pepsin was 0.5% (0.1 gram of product in 20 c.c. of water); the proportion of pepsin product in the presence of the collagen was 0.000,6%. A shortage of material necessitated the use of half portions of collagen (1 gram).

0	0.90	56	61	63	63	58	...	216	34		
0.3	0.90	64	73	86	95	115	...	182	68	34	3400
0.3*	0.90	53	59	60	61	59	...	216	34		

* The solution of pepsin added to the third mixture was taken from a portion of the stock solution which had been thoroughly *boiled* and then cooled to room temperature.

TABLE 2

Second series. Data showing the influence of trypsin on the absorption and retention of water by fibrin particles in dilute sodium carbonate solution containing sodium chlorid.

GENERAL DATA. The total volume of liquid in each test was 250 c.c. One fibrin preparation was employed throughout the series and the enzyme product was the same in each test ("Trypsin, Fairchild: 204").

Experiment 8. Weight of fibrin in each mixture: 6 grams. Concentration of sodium carbonate, 0.25%. Strength of the stock solution of trypsin, 2% (0.1 gram of product in 5 c.c. of water).

Content of enzyme and saline		Bulk of the sedimented fibrin mass (c.c.) at intervals during the progress of the experiment					Filtrate, c.c.	Amount of water absorbed		
Trypsin solution, c.c.	NaCl, per cent.	Hours						Total, c.c.	Excess over control	
		2	4	6	20	22			Absolute, c.c.	Ratio to fibrin, per cent.
0	0	93	105	113	122	122	227	23		
0.3	0	97	119	132	150	156	216	34	11	183
0	0.90	73	79	83	88	89	237	13		
0.3	0.90	75	83	92	107	110	232	18	5	83

TABLE 2 (continued)

Experiment 9. Weight of fibrin in each mixture: **6 grams.** Concentration of sodium carbonate, 0.25%. Strength of the stock solution of trypsin, 2% (0.1 gram of product in 5 c.c. of water).

Filtration of the liquid in the fourth mixture of the ninth experiment, because of its rapid digestion, was begun at the 27th hour, when its volume was 143 c.c.

Content of enzyme and saline		Bulk of the sedimented fibrin mass (c.c.) at intervals during the progress of the experiment							Filtrate, c.c.	Amount of water absorbed		
Trypsin solution c.c.	NaCl, per cent.	Hours								Total, c.c.	Excess over control	
		2	4	6	20	26	32	44			Absolute, c.c.	Ratio to fibrin, per cent.
0	0.90	77	80	82	87	89	90	92	234	16		
0.20	0.90	81	85	89	100	106	110	116	230	20	4	66
0.30	0.90	81	87	91	106	113	122	149	224	26	10	166
0.45	0.90	82	89	94	117	137	227	23	7	116

Experiment 10. Weight of fibrin in each mixture: **6 grams.** Concentration of sodium carbonate, 0.50%. Strength of the stock solution of trypsin, 1% (0.1 gram of product in 10 c.c. of water).

0	0.90	75	83	89	97	97	102	104	232	18			
0.2	0.90	87	93	96	111	117	123	129	230	20		2	33
0.3	0.90	88	95	99	117	126	132	142	225	25		7	116

Experiment 11. Weight of fibrin in each mixture: **3 grams.** Concentration of sodium carbonate, 0.25%. Strength of the stock solution of trypsin, 2% (0.1 gram of product in 5 c.c. of water).

Filtration of the fourth fluid in this series was begun at the 30th hour, when it was rapidly dissolving and its volume was 102 c.c. as recorded in the 32 hr. column; the first and third were filtered at the 32d hour; the second was filtered at the 45th hour, when its volume was 66 c.c. The fibrin in the fourth mixture dissolved completely during the process of filtration.

0	0.90	44	49	49	52	52	54	...	231	19			
0.1	0.90	45	51	52	58	61	64	68	234	16		-3	
0.2	0.90	46	52	53	65	72	80	...	227	23		4	133
0.3	0.90	45	51	54	70	89	102				

Experiment 12. Weight of fibrin in each mixture: **3 grams.** Concentration of sodium carbonate, 0.25%. Strength of the stock solution of trypsin, 2% (0.1 gram of product in 5 c.c. of water).

The solution of trypsin added to the third mixture was taken from a portion of the stock solution which had been thoroughly *boiled* and then cooled to room temperature.

0	0.90	39	42	43	48	50	236	14			
0.3	0.90	42	49	57	80	96	226	24		10	333
0.6	0.90	40	42	44	50	51	235	15			

tive hydrolysis which cannot be wholly avoided in experiments of this kind with pepsin and trypsin.

From the beginning of these studies it has been assumed that the recorded volume of sediment in experiments of this kind cannot

be a true measure of the amount of absorbed and retained water. The proteases effect a certain amount of cleavage as the experiment proceeds, the density and viscosity of the surrounding liquid increase with its progress, and the particles settle out less compactly as a consequence. The figure for bulk, as read from the calibration, is much greater, therefore, than the real value. On the other hand, since digestive solution takes place in such mixtures, the dissolved matter is lost from the particles. The amount of absorbed water as calculated from filtrate obtained is, accordingly, the quantity retained only by the undissolved portion of the original protein mass. These sources of obvious error influence the data in opposite directions. We have recorded the corresponding values obtained by both methods. The indications are seen to be identical in principle.

In Table I the differences between the 6 hr. and 15 hr. readings for mass of swollen *collagen* are usually much less than the differences between the 15 hr. and 18 hr. values. The sixth to fifteenth hours were the night hours of complete quiescence. We assume that cumulative imbibition of water occurred during that period without material effect on the relative positions of the swollen masses, but with diminution in the size of the interstices. After the mixtures were shaken in the morning, the gelatinous masses were not compressed by sedimentation into their previously more compact arrangement. The denser and firmer masses of *fibrin* were unlike those of the collagen in these respects. Exceptions to this rule were shown only by the fibrin masses that had been most markedly bloated and gelatinized under the influence of trypsin.

The results show clearly that proteases may increase considerably the absorption of water by protein particles immersed in acid or alkalin media containing proportions of sodium chlorid in excess of those that occur in protoplasm. Our data support the belief that hydrolases markedly influence the absorption, retention, accumulation and discharge of water by protoplasm, especially under abnormal conditions favorable to the development of edema.

Among the extensions of this work is a study, now in progress, of the behavior of *protoplasmic* masses in biological liquids under the influence of *intracellular* hydrolases.

DOCTORATES IN BIOLOGICAL CHEMISTRY

Conferred by American Universities, 1911-12

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:¹

Brown University.—*William Ward Browne*: Acid production by the *Bacillus coli* group.

Columbia University.²—*David 'Alperin*: Contribution to the knowledge of nucleoprotein metabolism, with special reference to uricolysis and to the properties of uricase.—*Louis Edward Bisch*: Biochemical studies of protagon and mucoid.—*Jacob J. Bronfenbrenner*: A biochemical study of the phenomena known as complement splitting.—*Alexander Oscar Gettler*: The balance of acid-forming and base-forming elements in foods, and its relation to ammonia metabolism.—*Marston Lovell Hamlin*: (1) The preparation of two derivatives of glucosamine; (2) Spigeline, an alkaloid of *Spigelia marilandica*; (3) Derivatives of 4-hydroxy-5-nitroquinazoline.—*Raleigh Frederick Hare*: A study of the chemistry of the carbohydrates of the prickly pear and its fruits.—*Max Kahn*: Biochemical studies of sulfocyanates.—*John Leonard Kantor*: A biochemical test for free acid.—*Chester Arthur Mathewson*: A study of some of the more important biochemical tests.—*Emily Cromwell Seaman*: Biochemical studies of the effects of beryllium sulfate.—*Harold Edward Woodward*: A study of surface tension of blood serum by the drop-weight method.

Cornell University.—*Harry Oliver Buckman*: Optimum and excessive soil moisture in its effect upon the soil and the crop.—

¹ In a few cases awards of the degree in *organic chemistry* (e. g., at Yale University), on subjects of special biochemical significance, are included.

² Additional information regarding the Columbia doctors (*and masters*) in biological chemistry, is given on page 575, where may also be found the names of successful Ph.D. candidates in botany, zoology and organic chemistry whose minor work was done, in part, in the Columbia department of biological chemistry.

Lewis Josephus Cross: A study of the relation of the chemical composition of hens' eggs to the vitality of the young chick.—*Merris Mickey McCool*: The antitoxic action of certain nutrient and non-nutrient mineral bases with respect to plants.—*Harry Westfall Redfield*: A study of hydrogen sulphide production by bacteria and its significance in the sanitary examination of water.—*John Edwin Turlington*: The effect on plant growth of nutrients applied at different periods.

Harvard University.—*Fred Ford Flanders*: The determination and metabolism of benzoic acid and hippuric acid.

Johns Hopkins University.—*Henry Otto Eysell*: (1) The detection of mannite in alkaline solutions of copper sulphate; decomposition of mannite by alkaline solutions of potassium permanganate in the presence of copper sulphate; (2) A determination of the volume of weight-normal solutions of cane sugar at 15°, 20°, 25° and 30°.

University of California.—*Charles Barrows Bennett*: The purines of muscle.—*Victor Birckner*: The oxidations and cleavages of glucose; yeast glucase, a new glucolytic ferment.—*Myrtle Elizabeth Johnson*: The control of pigment formation in amphibian larvae.—*Walter Pearson Kelley*: The functions and distribution of manganese in plants and soils.—*Edward Haslam Walters*: The hydrolysis of casein by trypsin.

University of Chicago.—*Warder Clyde Allee*: The effect of dissolved gases on the behavior of isopods.—*Melvin Amos Brannon*: The action of Salton sea water on plant tissues.—*Harry John Corper*: Correlation of chemical and histological changes in necrosis and autolysis.—*Sophia Hennion Eckerson*: A physiological and chemical study of after-ripening.—*Ernest Edward Irons*: Studies on immunity.—*Fred Conrad Koch*: The nature of the iodine complex in thyreo-globulin.—*Arno Benedict Luckhardt*: The relation of the spleen to the fixation of antigens and the production of immune bodies.—*Eugene Franklin McCampbell*: The toxic and antigenic properties of *Bacterium welchii*.

University of Illinois.—*Walter Edward Joseph*: A study of protein as a factor in the nutrition of swine, with special reference

to the distribution of the various forms of nitrogen in the animal body.—*Leonidas Rosser Littleton*: Molecular rearrangements in the camphor series; derivatives of isocamphoric acid; iso-aminolauronic acid and its decomposition products.—*Ellison Lloyd Ross*: Phosphorus metabolism of lambs.—*Albert Lemuel Whiting*: A biochemical study of nitrogen in certain legumes.—*Richard Hermon Williams*: A study of protein as a factor in the nutrition of swine, with special reference to the distribution of the forms of ash and phosphorus in the animal body.

University of Michigan.—*Harvey Clayton Brill*: A study of the formation of pyrimidines by use of nitromalonic aldehyde.—*Henry Newell Goddard*: Can soil fungi assimilate atmospheric nitrogen?

University of Wisconsin.—*Horace Grove Deming*: Some compounds of cellulose.—*Emil Oscar Ellingson*: On abietic acid and some of its salts.—*Winfield Scott Hubbard*: Studies of the tryptic digestion of silk.—*Alfred Edward Koenig*: A study of some of the salts of fatty acids.

Yale University.—*Charles Andrew Brautlecht*: Synthesis of thio-tyrosine.—*Gerald Burnham*: Sulphur combinations in proteins (thio-polypeptides).—*Amy Louise Daniels*: Fat-transport and metabolism, studied with the aid of fat-soluble dyes.—*Herbert Hartley Guest*: Thio-hydantoins and their biochemical interest.—*Charles Hoffman*: A new method for synthesizing alpha-amino acids; halogen derivatives of tyrosine.—*Robert Curtis Lewis*: The rate of elimination of nitrogen as influenced by diet factors.

Universities which conferred Ph.D. degrees in the natural and exact sciences, but which did not award the degree in biochemical science: Boston University, Bryn Mawr College, Catholic University of America, Clark University, George Washington University, Indiana University, Massachusetts Institute of Technology, New York University, Ohio State University, Princeton University, Stanford University, University of Cincinnati, University of Iowa, University of Minnesota, University of Pennsylvania, University of Pittsburgh, University of Virginia, Vanderbilt University, Washington University.

BIOCHEMICAL NEWS, NOTES AND COMMENT

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I. GENERAL

Necrology. *Dr. B. J. 'Austin*, lecturer in physiology and hygiene at University College, Reading, England, and latterly emeritus professor of botany.—*Professor Joseph von Bauer*, director of the first medical clinic at Munich, and author of many papers on physiology and therapeutics, particularly in the field of deranged metabolism and diseases of the blood vessels.—*Dr. Eugene Caven-
t**ou*, the distinguished organic chemist, president of the Paris Acad-
emy of Medicine in 1897.—*Dr. Edward Divers*, F.R.S., professor
emeritus of chemistry in the University of Japan.—*Dr. 'Alfred
Pribram*, professor of pathology in the German university at
Prague.—*Gustav L. Ramsperger*, honorary vice-president of the
New York College of Pharmacy.—*Dr. Ernst Schulze*, professor
of agricultural chemistry at the University of Zürich.—*Dr. Moritz
Seidell*, honorary professor of pharmacology at Jena.—*Prof. Ed-
u**ard Strasburger*, the eminent plant cytologist of the University of
Bonn.—*Judson B. Todd*, member of the N. Y. State Board of
Pharmacy since 1901.

In memoriam. *Lord Lister.* Steps have been taken by the
Royal Society in association with the Royal College of Surgeons
to organize a committee which will issue an appeal in behalf of a
formal memorial to the late Lord Lister.

Leonard P. Kinnicutt. At the Worcester Polytechnic Institute a fund has been established, to be called the *Leonard P. Kinnicutt Student Loan Fund*, with certain sums of money remaining after the discontinuance of Newton Hall, for eight years the institute dormitory on State St. Dr. Kinnicutt, while professor of chemistry, was chairman of the faculty committee in charge of the dormitory, and was always active in helping students financially and in other ways.

Robert Koch. The official *Reichsanzeiger* publishes the following edict from the Kaiser, dated March 29: "On March 24, 1912, thirty years had elapsed since the deceased Privy Councilor, Prof. Dr. Robert Koch, announced to the Berlin Physiological Society his discovery of the tubercle bacillus. With this discovery Koch initiated the battle against the severest scourge of the human race, and it has since then been carried on with unexampled success and has rendered to suffering humanity undying service. On this occasion I wish to honor the memory of the great scientist for all time by adding the name of Robert Koch to the title of the royal institute for infectious diseases in Berlin which was erected for Robert Koch and which was for twenty years his place of labor."

A memorial service for Robert Koch was recently held in Tokyo. The widow of Koch, the German ambassador and many scientists participated in the ceremony, which took place in the temple dedicated in honor of Koch by Professor Kitasato and which conformed to the Shinto ritual.

Howard T. Ricketts. The University of Chicago has received \$5000 from Mrs. Myra T. Ricketts, widow of the late Howard T. Ricketts, assistant professor of pathology in the university, to found a scholarship to be known as the *Howard T. Rickett's Prize*. This prize will be awarded annually for the best research by any student in the department of pathology and bacteriology. Dr. Ricketts lost his life in 1910 in the city of Mexico, from typhus fever, which he contracted while engaged in the scientific investigation of the disease.

Honors. Testimonials. The completion by Dr. Rudolf von Jaksch of twenty-five years as professor of internal medicine at

Prague was celebrated recently. The *Prager medizinische Wochenschrift* issued a special number in his honor and a *Festschrift* was presented to him.

On the completion recently of his fortieth year as a member of the faculty of the University of Berne, Prof. Theodor Kocher was given an ovation. The Swiss government, and universities and institutes, sent representatives, as did many of the European surgical societies. The *Deutsche Zeitschrift für Chirurgie* issued a special volume of 818 pages as a *Festschrift* in his honor. He celebrated the occasion by presenting to the university \$40,000 for the endowment of scientific research. It will be remembered that Kocher was awarded the Nobel prize in medicine, in 1909.

Ceremonies were conducted in the surgical amphitheater of the Hotel Dieu, Paris, on June 9, in honor of Dr. J. Lucas-Championnière, who introduced surgical antiseptics in France and who was recently elected a member of the Academy of Sciences. Professor Guyon presided. A commemorative medal, designed by Paul Richer, was presented to Dr. Lucas-Championnière. Addresses were delivered by M. Mesureur, Director of Public Charities, Professor Guyon and others.

On the retirement of Dr. Ira Remsen from the presidency of Johns Hopkins University, his colleagues of the various faculties presented to him a series of resolutions prefaced by eulogistic addresses.

The members of the Bureau of Chemistry presented to Dr. H. W. Wiley, as a farewell gift, a chest containing 144 pieces of flat silver, a massive meat platter with side dishes, and a porringer, paper spoon and cup for Harvey W. Wiley, Jr., born on May 16. The plate on the mahogany chest is inscribed as follows: "To Harvey W. Wiley, whose leadership has been an inspiration to all who have had the privilege of knowing personally, day by day, the breadth and depth of his well-stored mind, his unshakable integrity, and his splendid poise and never-failing geniality under any and all conditions. From the Bureau of Chemistry, U. S. Department of Agriculture, 1883-1912."

Awards of medals and prizes. The Franklin Institute of Pennsylvania has awarded an Edward Longstreth Medal of Merit to

Prof. Charles Baskerville, for his investigations on the chemistry of anesthetics (ethyl ether, chloroform, nitrous oxide and oxygen).

The third annual award of the Hunterian Society's medal has been made to Dr. A. Goulston, Heavitree, Exeter, for his essay on the use of sugar in heart disorders.

The second Willard Gibbs medal, founded by William A. Converse of Chicago, for distinction attained in chemistry, was presented to Prof. Theodore W. Richards by the Chicago section of the American Chemical Society, at a dinner on May 17. After the presentation, Professor Richards delivered an address on "Atomic weights."

Edward Longstreth Medals of Merit have been awarded to Drs. Oswald Schreiner and Elbert C. Lathrop in recognition of their important discoveries in agricultural chemistry. Dr. Milton Whitney, chief of the U. S. Bureau of Soils, has expressed the opinion that the results of the investigations which Dr. Schreiner has been conducting will revolutionize current methods for the enrichment of soils.

The Royal Society of Edinburgh has awarded to Professor Alexander Smith, the Keith Prize for the biennial period 1909-1911. The award was made for his researches on sulfur and on vapor pressure, which have been published in the *Journal of the American Chemical Society*.

Dr. Jokichi Takamine, who first prepared epinephrin in a commercial form ("adrenalin"), has been awarded a prize on that account by the Imperial Academy of Japan. The ceremony of presentation recently occurred in Tokyo.

Honorary degrees. Among the degrees conferred by the University of Michigan, at its recent celebration, was the doctorate of laws on Prof. W. H. Howell and the doctorate of science on Profs. John J. Abel and Henry Sewall.—Dr. H. W. Wiley received the degree of doctor of science from Lafayette College.

Membership in international scientific societies. Dr. Henry P. Armsby has been elected a foreign member of the Royal Academy of Agriculture, of Sweden.—Sir Victor Horsley, F.R.S., has been

elected to succeed Lord Lister in the Royal Society of Science, Upsala.—Dr. É. Metchnikoff has been elected foreign associate of the Académie des Sciences to succeed Sir Joseph Dalton Hooker, deceased.

Portrait presented. Some of the associates and students of Dr. Simon Flexner during the period from 1899 to 1904, when he was professor of pathology at the University of Pennsylvania, have presented to the university a portrait showing Dr. Flexner in his laboratory. The portrait was painted by Miss Adele Herter, of New York City.

Resignations and appointments. *Retirements.* Dr. Aristide Frébault, professor of chemistry and toxicology, and Dr. Edouard Maurel, professor of experimental pathology, have retired from the Faculté de médecine of Toulouse.

Prof. Armand Gautier has resigned the chair of medical chemistry in the University of Paris.

Mr. J. W. Hill, instructor in chemistry at the University of Montana, has resigned in order actively to develop distillation processes in the use of waste in lumbering. The new industry will be located at Bovill, Idaho.

Dr. James W. Holland recently resigned as dean, and professor of chemistry and toxicology, at the Jefferson Medical College. Dr. Holland was unanimously reelected dean and appointed professor emeritus of chemistry and toxicology. (See page 569.)

Prof. Max Schottelius has resigned the chair of hygiene at Freiburg in Baden.

Appointments. Dr. W. M. Bayliss, F.R.S., has recently been appointed university professor of general physiology in University College, London.

Dr. Edward H. Bradford was elected dean of the Harvard Medical School, vice Dr. Henry A. Christian, resigned (page 491).

Dr. Charles A. Brautlecht, a graduate student at Yale, has been appointed instructor in chemistry and physics at Throop Polytechnic Institute, Pasadena, Cal.

Dr. Frederick C. Busch has resigned as professor of physiology in the University of Buffalo to accept the position of clinician at the New York Institute for the Study of Malignant Diseases, and has been succeeded by Dr. Frederick H. Pratt.

Mr. Mark A. Carleton, for the past eighteen years in charge of grain investigations in the Bureau of Plant Industry, and well known as the introducer and propagator of Durum wheat and the Swedish select oat, has resigned his present position to take charge of the work of the Pennsylvania Chestnut Tree Blight Commission.

Dr. R. C. Collison of the department of nutrition, at Ohio Agricultural Experiment Station, has resigned to accept the position of field expert, in the department of soils, at New York Agricultural Experiment Station.

Dr. C. E. Craig, instructor in agronomy in Purdue University, has accepted the position of agronomist in the Polytechnic School at Porto Alegre, Brazil.

Dr. L. J. Cross has been promoted from instructor to assistant professor of agricultural chemistry at Cornell University.

Prof. Friedrich Czapek, of the University of Prague, has been appointed to the chair of plant physiology and pathology in the Imperial College of Science and Technology, London.

For the chair of medical chemistry, in the Faculté de médecine of Paris, to succeed Prof. Armand Gautier, who recently retired, the choice fell on Dr. Desgrez, *agrégé* at the school and for many years a co-worker with Professor Gautier.

Prof. B. M. Duggar, of Cornell University, has been elected to the professorship of plant physiology and applied botany in Washington University, vacated by Dr. George T. Moore in accepting the directorship of the Missouri Botanical Garden (page 492).

Dr. Dürck, until a short time ago director of the pathologic institute at Jena and for the last few months prosector of the pathologic institute of a municipal hospital in Munich, has assumed the direction of the pathologic institute at Rio de Janeiro.

Dr. David L. Edsall has been elected Jackson professor of clinical medicine in Harvard Medical School, vice Dr. Frederic C. Shattuck, resigned.

Mr. C. Fritz, a recent graduate of Ohio State University, has been appointed analyst, in the department of nutrition, at Ohio Agricultural Experiment Station.

Dr. J. Reynolds Green has been appointed Hartley lecturer in vegetable physiology in the University of Liverpool.

Prof. F. O. Grover, head of the department of botany in Oberlin College, has been appointed by the faculty to represent the college in the Ohio Biological Survey.

Dr. Howard D. Haskins has been promoted to an associate professorship of organic chemistry and biochemistry in Western Reserve University.

Mr. James Hendrick, lecturer in chemistry at Aberdeen Agricultural College, has been appointed professor of agriculture in the university.

Dr. Thomas F. Hunt, director of the School of Agriculture of Pennsylvania State College, has been appointed to succeed Dr. E. J. Erickson, dean of the College of Agriculture in the University of California.

Mr. F. W. Jones has been appointed chief chemist of the purification works of the Fitchburg, Mass., Sewer Department. Mr. Jones was for some time instructor in chemistry at the Worcester Polytechnic Institute. During the past year he has been assistant chemist at the Worcester sewage purification works.

Dr. R. W. Keeton, of the University of Illinois, has been appointed adjunct professor of experimental physiology and physiological chemistry in Albany Medical College.

Dr. Arthur I. Kendall has been given charge of the research on tuberculosis in Northwestern University, the chair for which was recently endowed with \$250,000 by James A. Patten, Evanston, Ill.

Miss Janet Lane-Claypon, lecturer on hygiene and physiology at Battersea Polytechnic Institute, has been appointed lecturer on hygiene and physiology at King's College for Women, London.

Dr. R. N. Lyne, director of agriculture in Portuguese East Africa, has been appointed director of the new agricultural department of Ceylon.

Dr. Charles E. Marshall, professor of bacteriology and hygiene in the Michigan State Agricultural College, has accepted the directorship of the graduate school, and the professorship of microbiology, at the Massachusetts Agricultural College.

Dr. J. W. Nowell and Mr. F. B. Sherwood have been appointed instructors in chemistry in the North Carolina College of Agriculture and Mechanic Arts.

Dr. Eugene L. Opie, professor of pathology in Washington University, has been appointed dean of the medical school, vice Dr. George Dock, retired from this position at his own request.

At the University of Wisconsin, Dr. James B. Overton has been promoted from assistant professor to associate professor of plant physiology; and Mr. Gilbert M. Smith from assistant to instructor in botany.

Prof. Thomas L. Patterson, head of the department of biology in the Highland Park College, has accepted an appointment as associate professor of biology and physiology in the University of Maryland School of Medicine.

Dr. William Pepper, professor of clinical pathology at the University of Pennsylvania, son of a former provost of the university, has been appointed dean of the Medical Department, to succeed Dr. Allen J. Smith, resigned. Dr. Smith will remain professor of pathology, comparative pathology and tropical medicine.

Dr. Amos W. Peters, of the Carnegie Nutrition Laboratory, has been appointed biochemist in the research department of the Training School for Feeble Minded Children, at Vineland, N. J.

Dr. E. J. Russell, at present Goldsmiths' Company's assistant in soil investigations, has been appointed director of the Rothamstead Experimental Station, in succession to Mr. A. D. Hall, F.R.S.

Dr. DeWitt H. Sherman has succeeded Dr. Eli H. Long as professor of materia medica and therapeutics in the University of Buffalo.

Dr. Julius W. Sturmer, professor of pharmacy at Purdue University, has been elected to the chair of pharmacy and to the deanship for pharmacy, at the Medico-Chirurgical College, Philadelphia, vice Prof. I. V. S. Stanislaus, resigned.

Dr. Francis W. Upshur has been appointed professor of materia medica and therapeutics, and Dr. C. Howard Lewis, professor of pharmacology and also associate professor of physiology, in the College of Medicine of the University of Virginia. These two physicians divide the chair which was formerly held by Dr. Virginius Harrison. Dr. E. C. L. Miller was elected professor of bacteriology and physiological chemistry at the same institution.

Dr. E. de Wildman has been appointed director of the Brussels Botanical Garden.

Dr. Francis Carter Wood has been appointed director of cancer research at Columbia University on the George Crocker Foundation (page 561).

Promotions and appointments at the Rockefeller Institute for Medical Research.—Alexis Carrel, associate member in experimental surgery, promoted to the rank of *member* of the institute; associates made *associate members* for a term of three years: Peyton Rous, pathology and bacteriology, Donald D. Van Slyke, chemistry, Walter A. Jacobs, chemistry, and Frank W. Bancroft, experimental biology; assistants made *associates*: Paul F. Clark, pathology and bacteriology, Richard V. Lamar, pathology and bacteriology, and Hardolph Wasteneys, experimental biology; *new appointments*: Harold L. Amoss, assistant in pathology, Clarence J. West, assistant in chemistry, Wolfgang Ewald, fellow in experimental biology, Francis R. Fraser and Frederic M. Hanes, assistant resident physicians and assistants in medicine.

Officers-elect of biological organizations. The names of recently selected officials of important biological organizations are grouped below.

AMERICAN MEDICAL ASSOCIATION—GENERAL OFFICERS: *President*, John A. Witherspoon; *Vice-Presidents*, P. A. Harris, J. L.

Heffron, H. H. McClanahan and H. D. Fry; *Secretary*, Alexander R. Craig; *Treasurer*, W. A. Pusey; *Trustees*, M. L. Harris, C. A. Daugherty and W. T. Councilman; *Member of the Judicial Council*, G. W. Guthrie; *Member of the Council on Health and Public Instruction*, W. B. Cannon; *Members of the Council on Medical Education*, J. W. Holland and W. D. Haggard.—SECTION ON PATHOLOGY AND PHYSIOLOGY: *Chairman*, A. W. Hewlett; *Vice-Chairman*, A. J. Carlson; *Secretary*, William Ophüls; *Executive Committee*, Yandell Henderson, H. G. Wells and Leo Loeb; *Delegates*, Yandell Henderson and (alternate) J. W. Vaughan.—SECTION ON PHARMACOLOGY AND THERAPEUTICS: *Chairman*, R. L. Wilbur; *Vice-Chairman*, J. F. Anderson; *Secretary*, M. I. Wilbert; *Executive Committee*, D. L. Edsall, Lawrence Litchfield and Torald Sollmann; *Delegates*, Torald Sollman and (alternate) Reid Hunt.

ASSOCIATION OF AMERICAN PHYSICIANS: *President*, L. F. Barker; *Vice-President*, Simon Flexner; *Secretary*, G. M. Kober; *Recorder*, S. Solis Cohen; *Treasurer*, J. P. C. Griffith.

AMERICAN ASSOCIATION OF MEDICAL MILK COMMISSIONS: *President*, O. M. Edwards; *Secretary*, O. P. Geier; *Treasurer*, S. M. Hamill; *Councillors*, T. C. McCleave and J. R. Williams.

AMERICAN ASSOCIATION OF PHARMACEUTICAL CHEMISTS: *President*, H. A. Stiles; *Vice-Presidents*, G. C. Hall and E. T. Warnock; *Secretary-treasurer*, W. P. Stearns.

NATIONAL ASSOCIATION OF MANUFACTURERS OF MEDICINAL PREPARATIONS: *President*, F. G. Ryan (Parke, Davis and Co.); *Vice-President*, A. G. Rosengarten (Powers-Weightman-Rosengarten Co.); *Secretary*, C. M. Woodruff (Parke, Davis and Co.); *Treasurer*, H. C. Lovis (Seabury and Johnson); *Executive Committee*, A. R. L. Dohme (Sharp and Dohme), C. J. Lynn (Eli Lilly and Co.), and the officers.

MEMBERSHIP OF THE COMMITTEE ON OCCUPATIONAL DISEASES IN CHEMICAL TRADES, *New York Section of the American Chemical Society*: Prof. Charles Baskerville, College of the City of New York, *Chairman*; Dr. Geo. P. Adamson of Baker and Adamson Chemical Co.; Mr. W. H. Bassett of American Brass Co.; Dr. Wm. F. Doerflinger, consulting chemist; Dr. H. M. Kauffman of Mutual

Chemical Co. of America; Dr. A. C. Langmuir, Chairman New York Section of the Amer. Chem. Soc.; Dr. Geo. A. Prochazka of Central Dye Stuff and Chemical Co.; Dr. Geo. D. Rosengarten of Powers, Weightman and Rosengarten; Dr. A. H. Sabin of National Lead Co.; Dr. Charles L. Parsons, U. S. Bureau of Mines; Mr. E. C. Uhlig, *Secretary*, Brooklyn Union Gas Co.

MEMBERSHIP OF THE COMMITTEE ON INDUSTRIAL DISEASES, *New York Association for Labor Legislation*: Prof. H. R. Seager, President American Association for Labor Legislation, *Chairman*; Prof. S. McC. Lindsay, President N. Y. Association for Labor Legislation, *ex officio*; Dr. C. L. Dana, Chairman Committee on Public Hygiene, N. Y. Academy of Medicine, *ex officio*; Dr. J. B. Andrews, Secretary American Association for Labor Legislation; Profs. Charles Baskerville and C. E.-A. Winslow, College of the City of New York; Drs. Warren Coleman, J. H. Huddleston, J. Alex. Miller, W. G. Thompson and L. R. Williams, N. Y. Academy of Medicine; Messrs. M. M. Dawson, Actuary; L. W. Hatch, Statistician Department of Labor State of New York; F. L. Hoffman, Statistician Prudential Life Insurance Co.; and Mr. Paul Kennaday, *Secretary*, N. Y. Association for Labor Legislation.

Meetings. *American Medical Association.* The sixty-third annual session of the American Medical Association was held at Atlantic City, June 3-7. The retiring president, Dr. Abraham Jacobi, delivered an address on "The best means of combating infant mortality."—"In view of the very superior excellence of his research exhibit on experimental nephritis," a gold medal was awarded, by the Committee on Awards, to Prof. Martin H. Fischer.—At a session of the Section on Pharmacology and Therapeutics, the following resolution was adopted: That the Chairman appoint a committee of three to draw up resolutions to be presented by the representative of the Section on Pharmacology and Therapeutics to the House of Delegates, to attempt to secure legislation forbidding patents of materia medica articles and permitting patents only of process of manufacture; and to request the manufacturers to act in coöperation with the Association in this necessary legislation.

Fifteenth International Congress on Hygiene and Demography. Thirty-two foreign countries have accepted the federal govern-

ment's invitation to participate in the Fifteenth International Congress on Hygiene and Demography in September, and lists of official delegates have already been received from twenty-five. These lists contain many names which are well known, both to the medical profession and to sanitarians in general, as foremost authorities in their particular lines of endeavor.—Prof. Max Rubner, director of the Physiological Institute of the University of Berlin, will deliver one of the principal addresses.—The Fifteenth International Congress on Hygiene is a powerful instrument for permanently improving sanitation in this country. The new and permanent impetus which the congress may confidently be expected to give to progressive work in every branch of hygiene in America is eagerly awaited by all who are interested in civic welfare and public health.

Association of specialists in digestive and metabolic diseases. On May 27 a meeting for the organization of specialists in digestive and metabolic diseases was held at Hamburg, in which seventy German specialists participated. It was decided to hold meetings yearly, but it was expressly agreed that no separation from the Congress for Internal Medicine is intended. Drs. Ewald of Berlin, Schmidt of Halle, Boas of Berlin, Weintraud of Wiesbaden, Starck of Carlsruhe and Pariser of Homburg will be the first executive committee. The initial scientific meeting will be held after the annual session of the *Naturforscherkongress* in September, 1913, at Homburg.

Buildings, funds and general equipment. *Buildings.* An anonymous donor has given 10,000 guineas for the erection of a physiological laboratory at the medical school of University College of South Wales and Monmouthshire.—An addition is being made to the agricultural building of the University of Illinois by enclosure of the court. The structure will be one story high, with cement floors, and will provide reading room, class rooms, museum, etc.—The University of Missouri is erecting a building for the department of chemistry, mainly for agricultural chemistry, at a cost of \$60,000. This building has been named Schweitzer Hall in memory of Prof. Paul Schweitzer who was for nearly forty years connected with the department.—The new medical laboratories for the two years' course in medicine at the University of North Carolina were

opened on May 8.—Agricultural hall, recently erected by the University of California at a cost of \$200,000, is ready for occupancy.

Endowments. Western Reserve University has completed its endowment fund of \$1,000,000 for the medical school, \$250,000 of which was contributed by Mr. John D. Rockefeller.—A chair of agriculture will be established in the University of Queensland, toward which Mr. Robert Philp has offered to give £1,400 and Mr. R. M. Christison £1,000.—Appropriation bills in behalf of the College of Agriculture, Cornell University, to the amount of \$907,000, of which \$788,000 is immediately available, were passed by the New York legislature at its recent session and signed by the Governor.—The bill appropriating \$250,000 for the development of work in public health and medicine at the University of Illinois, failed of passage in the special session of the Illinois legislature.

A new Pasteur Institute. The Pasteur Institute, for the manufacture of virus to be used in combating rabies, is the latest addition to the scientific laboratories of the University of California. Dr. W. A. Sawyer is the director.

Cancer research. With the appointment of Prof. F. C. Wood to the position of director of cancer research at Columbia University on the George Crocker Research Foundation, the final step has been taken in the organization of the work which will be conducted with this great gift. The construction of the laboratories and the organization of the staff will probably consume another year, so that it will be some time before effective research will be under way. This appointment will add certain further resources of great advantage to the Crocker fund, for Professor Wood will continue as director of the laboratories of St. Luke's Hospital, where he has a clinical service for the scientific study of special cases. This will permit the workers on the Crocker foundation to investigate, with speed and certainty, the claims of alleged cancer cures; and if any of the Columbia investigators discover a remedy for cancer, the necessary preliminary tests could easily be made in Professor Wood's service in St. Luke's.

The endowment fund of the General Memorial Hospital has been increased by a contribution of \$100,000, from a scientist whose

name has not been made public, for the maintenance of twenty beds for cancer patients. The hospital was chartered for the study and treatment of cancer and allied diseases, and is supported by the Collis P. Huntington fund. This institution has been affiliated with Cornell Medical College and a great deal of research has been done. Abundant opportunity will be given to trained men for the study of cancer at the bedside and, for the first time, New York has an institution similar to those established in Berlin, Heidelberg, Paris, London, Boston, Buffalo and St. Louis.

Radium notes. *Radium bed unde: Budapest.* Dr. Wesselsky, professor of chemistry at Budapest University, believes that there are vast radium strata under Budapest and that the high temperature and healing virtues of the Budapest thermal springs are due to the radium in these strata. Dr. Wesselsky has found that the thermal springs of Budapest are five or six times as rich in radium as the most celebrated foreign springs. According to him, the water of the Budapest springs contains 35 mg. of radium in every 10 liters of water.

Opening of a new radium station in the Vienna General Hospital. A very important addition to the therapeutic armamentarium of the Vienna General Hospital has been made by the opening of a radium institute as an annex to the dermatologic clinic of Prof. Gustav Riehl. This "radium station" is intended primarily for the treatment of patients in the hospital but it will also be open to medical practitioners outside the hospital, to whom a number of "radium cells" will be loaned and water charged with emanation will be sold. The station owns half a gram ($7\frac{1}{2}$ grains) of pure radium compound, valued at about \$50,000, which has been presented by the ministry of public works from the radium establishment in Joachimstahl (page 509). The "station" will be self-supporting and any net profit will be devoted to its enlargement. A special research department will soon be added.

Radium institute for biologic and therapeutic research in Berlin. The institute founded by the efforts of Prof. Wilhelm His for biologic and therapeutic research on radium in the royal Charité was opened recently. The purpose of the institute is the investigation of

the therapeutic effects of radio-active substances such as radium, mesothorium, radiothorium, etc., and their decomposition products. It possesses laboratories for chemical, zoological and botanical research, and a complete equipment for physical measurements. The institute has been founded solely by private enterprise, contributions to the extent of \$5,000 a year having been guaranteed by the Kaiser Wilhelm Gesellschaft zur Förderung der Wissenschaften and by two radium firms. The two industrial firms are also prepared to furnish the necessary material and measuring apparatus. The institute has not been established in a new building. It is installed in a rented house in Luisenplatz, in the neighborhood of the Charité, where it is connected with a polyclinic in which internists, surgeons and other interested specialists will have opportunity to treat patients with radiation apparatus of various forms and strength, emanators for inhalation in closed rooms, apparatus for drinking the emanations, and other forms of application. The director of the institute is privy councilor Wilhelm His, whose representative is Dr. Gudzent. He is supported by a commission to which Professors Kraus, Orth, Lesser, Hildebrand, Bier, Zuntz, Hertwig, Hahn and Marckwald have assured their coöperation. Fifteen work places are provided in the laboratory, which are accessible to foreign physicians. There are two assistants to the director of the institute. A permanent physico-chemical collaborator has been secured.

Journalistic. *New Bulletin.* Eli Lilly and Co., of Indianapolis, have begun the publication of *The Lilly Scientific Bulletin*. The first number was issued on April 16. "Upon request, the Lilly Scientific Bulletin will be sent regularly to libraries and individuals interested in the sciences related to medicine and pharmacy."

A new journal for chemotherapy. Few branches of medicine have experienced a development as sudden, intensive and effective as the new domain of chemotherapy associated so closely with the name and efforts of Paul Ehrlich. With mushroom-like growth, new principles in therapy and new procedures in diagnosis have arisen, commanding attention in every land where modern scientific medicine exerts its beneficent influence. The literature which the new work and its exploitation has inspired in a period counted better by months than years, now reaches enormous proportions. This has

led to the establishment of a new journal intended for laboratory investigator and practitioner alike. With the title of *Zeitschrift für Chemotherapie und verwandte Gebiete* (Verlag von Georg Thieme, Leipsic), the new publication is edited under the supervision of Profs. Paul Ehrlich, Friedrich Kraus and August v. Wassermann—names which serve to give the journal an immediate prestige in the field of immunotherapy and diagnosis. It is planned to publish original articles at indefinite intervals, while the review features, forming a continuation of the *Folia Serologica*, will appear regularly. The first number of 200 pages is devoted to salvarsan (606) therapy, and includes elaborate reports from Germany, Great Britain, France, Italy, Russia and the United States. The American review is by Dr. John A. Fordyce of New York.

Miscellaneous items. *Change of name.* Prof. Carl Fraenkel, director of the hygienic institute of Halle, has changed his name to Fraenken with the consent of the government.

The *Harrington lectures* at the University of Buffalo were delivered on May 28–31, by Dr. Ludvig Hektoen, subject, “Immunity.”

Visiting agricultural commission. The consul general of Uruguay and six members of a commission on agricultural engineering are visiting our colleges of agriculture. The commission is on a world tour for the study of agriculture in different countries.

“*Phossy jaw*” bill signed. The bill taxing white phosphorus matches has become a law. It is expected that this law will prevent the use of white phosphorus in the manufacture of matches.

Sanitary survey. Prof. George C. Whipple, Dr. J. W. M. Bunker and Mr. M. C. Whipple, of Harvard University, are making a sanitary survey of Lake Ontario near the mouth of the Genesee River, in order to ascertain the effect of the sewage of the city of Rochester on the river and lake.

Prohibition of absinthe. A senatorial committee has unanimously decided that the essence of thujone should not be used in any liquor, that the nomenclature of plants containing thujone should be officially established, and that traffic in such plants should

be forbidden. Two years' grace will be allowed the manufacturers of absinthe for the disposal of present supplies and three years' grace to the dealers. Consumers are presumably committed, meanwhile, to the grace of God.—The National Pure Food Board has ruled that on and after Oct. 1 the importation of absinthe will be illegal.

Health board appointment declined. Dr. H. W. Wiley has declined proffered appointment to the chairmanship of the Board of Health of Boston. In a letter to Congressman Murray of Massachusetts, Dr. Wiley said, in this connection: "I could not contemplate placing myself again in the condition through which I passed in the last few years. My idea of a health officer is that he should, within the statute, have unrestricted freedom of action, and be responsible for the results."

Medical school to be independent. The affiliation which has existed for fifteen years between the College of Physicians and Surgeons of Chicago and the University of Illinois has been dissolved. This action resulted from the initiative of the medical faculty through its refusal to renew the lease of the school to the university. It was concluded that unless the university could provide for its medical school as it does for its other departments, it would be better for the college to pursue an independent course along the lines of the policy already established.

Medical research. No medical school can be fully alive to the present needs of medical education unless it has the constant inspiration that comes through medical research within the college. How can the school without research know what subjects should be most emphasized in the medical curriculum, or what methods should be employed in teaching? Nevertheless, we have in this country today only thirty medical colleges where active and valuable research is being carried on. In about thirty others some little effort at research is being made by individuals, but the men are handicapped by lack of time, assistants or material, or the college is of too low a standard to receive any benefit from it. *In at least fifty-six medical colleges no research work whatever is being carried on.* (Report of the Council on Medical Education of the American Medical Association: *Journ. Amer. Med. Association*, lviii, p. 1795: June 8).

Resuscitation after electric shock. The Commission on Resuscitation after electric shock (page 495), organized on the initiative of the National Electric Light Association, and having for its purpose the study of electric shock and the preparation of rules for first aid in cases of electric accident, has prepared the results of its work for presentation to the public. There is a simple chart to be posted in all factories and electric shops, and a more detailed set of rules embodied in a sixteen-page booklet to be carried in the pocket of the electrician. These represent the commission's findings on the question of producing artificial respiration in emergency cases. The commission will also publish a report on mechanical means of producing artificial respiration, and it will present its judgment on the insufflation method advocated by Dr. S. J. Meltzer.

Synthetic rubber. Prof. W. H. Perkin lately read a paper before the Society of Chemical Industry, announcing that rubber has been synthesized and that synthetic rubber can be placed on the market at a price to compete with plantation rubber. In 1909 Mr. E. Halford Strange, of Messrs. Strange and Graham, technical research chemists, directed his organization of chemists, headed by Dr. F. E. Matthews, to the problem of the synthetic production of rubber. In July, 1910, Dr. Matthews left some metallic sodium in contact with *isoprene*, and in the following September found that the isoprene had turned into a solid mass of rubber. On further investigation it was found that sodium is a general polymerizing agent for such substances. The first *announcement* of this discovery was made by Prof. Carl Harries, of Kiel University, who made the same discovery independently, about three months *later*. Dr. Matthews suggested a method for preparing isoprene in which *acetone* was one of the raw materials, and, later, one in which *fusel oil* was the starting point. Professor Perkin was then asked to coöperate. Subsequently Sir William Ramsay joined the group as consultant. Professor Fernbach, of the Pasteur Institute, after eighteen months of laborious work, discovered a *fermentation process* for the commercial production of fusel oil from starchy materials. This process is now so satisfactory that the higher alcohols can be obtained at a cost of not more than £30 per ton, thus providing cheap material in abundance for rubber synthesis.

II. LABORATORY FOR PATHOLOGICAL CHEMISTRY AT THE NEW YORK POST-GRADUATE MEDICAL SCHOOL AND HOSPITAL

For the past several years the New York Post-Graduate Medical School has been endeavoring to enhance its clinical advantages and general equipment and, so far as possible, to raise the plane of post-graduate medical teaching. The laboratories for tropical medicine, bacteriology, pathology and pathological chemistry, of which Prof. Jonathan Wright (M.D., Columbia '83) is the Director, have been a part of this general scheme.

The laboratory for pathological chemistry, which was opened in April, has been fully equipped for teaching, for the routine examination of hospital specimens, and for original research. Courses are offered at scheduled intervals, first, to satisfy those physicians who wish to become familiar with the modern chemical methods of diagnosis; second, to meet the demand of such as can spend a greater amount of time in the laboratory and desire to learn the general methods and development of our modern physiological and pathological chemistry. In addition, a course is offered in the physiology and pathology of metabolism and nutrition. The intimate connection with the hospital and the large number of specimens daily received at the laboratory, furnish excellent material for these courses.

Thirty-five matriculates have availed themselves of these courses since October. Victor C. Myers, M.A. (Wesleyan '07), Ph.D. (Yale '09), is professor of pathological chemistry and Morris S. Fine, Ph.D. (Yale '11), instructor in pathological chemistry. In addition, the personnel of the laboratory is composed of the two internes assigned to this laboratory for the first two of their six months' laboratory service, together with the laboratory technician. During the summer, G. O. Volovic, formerly assistant in physiological chemistry at the Albany Medical College, will aid in the investigation of special research problems.

III. DEPARTMENT OF BIOLOGICAL CHEMISTRY OF THE STATION FOR EXPERIMENTAL EVOLUTION: THE CARNEGIE INSTITUTION OF WASHINGTON

The work of the Bio-chemical Research Laboratory of the Station for Experimental Evolution, at Cold Spring Harbor, Long

Island, was inaugurated in the fall of 1909 with Doctor R. A. Gortner as the bio-chemist in charge. The breeding experiments which had been conducted at the Station prior to 1909 had necessitated the postulating of theories regarding bio-chemical processes. The laboratory was installed in order to test the validity of these hypotheses.

A temporary laboratory was fitted up in one of the rooms of the main building but recently more space was needed and a wing of a new building has been built in which there are five rooms devoted to the work of the bio-chemist. These consist of a large, well lighted laboratory, a weighing room and office combined, an operating room, a sterilizing room, and a room containing cages where animals may be kept and the effect of various chemicals or secretions on them or their offspring may be determined.

Since the laboratory has been established the main subject under investigation has been the origin and nature of the animal pigments, or melanins. Some of the earlier ideas regarding the nature of melanin have been proven erroneous, especially the idea of their "indestructibility" by chemical reagents. It has also been found that the term melanin may include two or more distinct types of compounds. Twelve papers have been published by Dr. Gortner since the establishment of the laboratory, as follows:

1910. A contribution to the study of the oxidases. *Trans. London Chem. Soc.*, 97, 110-120.—The origin of the brown pigment in the integuments of the larva of *Tenebrio molitor*. *Journ. Biol. Chem.*, 7, 365-70.—Spiegler's white melanin" as related to dominant or recessive whites. *Amer. Naturalist*, 44, 497-502.—Studies on melanin. I. Methods of isolation. The effect of alkali on melanin. *Journ. Biol. Chem.*, 8, 341-63.

1911. A new decomposition product of keratin which gives Millon's reaction. *Ibid.*, 9, 355-7.—Studies on melanin. II. The pigmentation of the adult periodical Cicada, *Tibicen septendecem* L. *Ibid.*, 10, 89-94.—Studies on melanin. III. The inhibitory action of certain phenolic substances upon tyrosinase. A suggestion as to the cause of dominant and recessive whites. *Ibid.*, 10, 113-122.—Studies on melanin. IV. The origin of the pigment and

the color pattern in the elytra of the Colorado potato beetle, *Leptinotarsa decemlineata*, Say. *Amer. Naturalist*, 45, 743-55.—On melanin. *BIOCHEMICAL BULLETIN*, 1, 207-15.

1912. Sur les pigments mélaniques d'origine animale. *Bull. Soc. Chim.*, 11, 498-500.—The occurrence, and the significance, of tyrosinase in the reproductive organs of certain amphibians. *Proc. Soc. Exper. Biol. Med.*, 9, 118-120.—On two different types of melanin. *Ibid.*, 9, 120-1.

IV. COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

I. General notes

Marriages: On February 24, Miss Isabelle Mary Shoolbred and Dr. Elmer W. Baker.—On July 3, Miss Edith Downs and Dr. George D. Beal.

Appointments and promotions. Prof. P. B. Hawk has resigned his chair at the University of Illinois to accept appointment to the professorship of physiological chemistry at Jefferson Medical College, vice Dr. J. W. Holland resigned (page 553).

For the more complete care of the health of the students, particularly the undergraduates in residence, Dr. William H. McCastline, now assistant professor of physical education, has been appointed health and sanitary officer of Columbia, from July 1. Dr. McCastline will have responsible charge, as health and sanitary officer, of the general health and sanitary conditions at Columbia and will be accessible for daily consultations with students, who, while not needing clinical treatment, would be benefited by medical advice and oversight.

Prof. Frederic M. Hanes, who was recently promoted from associate to assistant professor of pathology at Columbia, has accepted appointment as resident physician and assistant in medicine at the Rockefeller Institute (page 557).

Prof. Clarence E. May has been promoted from assistant professor to associate professor of chemistry at the University of Indiana.

Dr. Harry L. Fisher has resigned his instructorship in organic chemistry at Cornell Medical College to accept a similar position in the chemical department at Columbia (page 574).

Miscellaneous notes. We have received a copy of the handsomely illustrated pamphlet on "The Daniel Baugh Institute of Anatomy of the Jefferson Medical College of Philadelphia," containing a history of its foundation," a "description of the building," a "statement of its adaptability to teaching anatomy" and an account of the dedication exercises," with floor plans, etc. Anatomy at Jefferson Medical College has always flourished and has been abreast of the times from the days of Nathan Smith, its first professor of anatomy, through the periods of his worthy successors, George and Samuel McClellan, Granville Sharp Pattison, Joseph and William Pancoast, and William S. Forbes, to the present era under the leadership of the distinguished director of the new institute, Prof. Edward Anthony Spitzka.

Dr. William N. Berg has lately been engaged in a study of the digestibility of immature veal, i. e., veal less than three weeks old. It is illegal to sell such veal and the object of the work is to find out, if possible, whether there is any other than an esthetic justification for the law.

Dr. C. C. Lieb has been elected an active member of the Harvey Medical Society.

The Ph.D. degree was recently conferred upon Fred J. Seaver by Iowa State University.

2. Proceedings of the Association.

Informal lecture at the College of Physicians and Surgeons. *Fifth scientific meeting* (page 514). On May 1, Dr. Paul E. Howe, of the University of Illinois, delivered, at the invitation of the Association, a very interesting and instructive lecture on "Fasting," to the members and their guests including the class of first year medical students. An abstract of the lecture will be published in the succeeding issue of the BIOCHEMICAL BULLETIN. (See page 574).

Proceedings of the sixth scientific meeting (page 514). *Third annual meeting.* The third annual meeting of the Associa-

tion, which was held at the College of Physicians and Surgeons on the evening of June 3, brought together a large number of members and guests, who again enjoyed a long and very pleasant session. In the unavoidable absence of President H. O. Mosenthal (abroad) and Vice-President Jacob Rosenbloom (out of town), Professor Gies called the meeting to order, extended personal greetings to all in attendance and proposed that Dr. Walter H. Eddy be requested to preside. This proposal was enthusiastically ratified and Dr. Eddy conducted the proceedings with his accustomed grace and efficiency.

The proceedings consisted of research communications by members of the Association, followed by a business session. There were nearly fifty titles on the scientific program, but, as only about fifteen members could present oral reports in the allotted time, the remainder were read by title. Abstracts of the communications comprising the scientific proceedings will be published in group in the September issue of the BULLETIN.

The business session was conducted informally while the members did justice to refreshments. Professor Gies discussed the gratifying history of, and the promise of continued future usefulness for, the BULLETIN. He announced that members and friends of the Association had contributed sufficient funds to enable the editorial committee to pay all expenses thus far. He expressed the hope that the BULLETIN would retain its present sturdy support, which far surpassed his most sanguine expectations, and that it would steadily gain new subscribers, so that the subscription price could be made even less than it is or the number of pages per volume considerably increased. Professor Gies emphasized the professional utility of the BULLETIN and laid special stress on its function of service to chemical biology. He felicitated the Association on the exceptional opportunity in this regard which the BULLETIN afforded to it. He suggested the appointment of a Board of Directors for the executive conduct of BULLETIN affairs during the intervals between meetings of the Association, the Editorial Committee to serve under the directors. His proposal to this effect was based on the belief that a Board of Directors would stabilize the management of the

BULLETIN in all emergencies and strengthen its growth besides. This suggestion, when put to a vote, was unanimously approved.

On nomination by Professor Welker, Dean Samuel W. Lambert was unanimously elected an Honorary Member. On nomination by Dr. Alfred P. Lothrop, Prof. Alexander Smith was unanimously elected an Honorary Member. Professors Chittenden (page 337), Lambert and Smith now constitute the honorary membership of the association.

At the suggestion of Professor Gies the association decided, by unanimous vote, to invite Dr. Jacques Loeb to be the guest of honor at the second annual dinner of the Association next fall. Dr. Loeb has graciously accepted the invitation, a committee of arrangements has been appointed, and a very enjoyable time may be anticipated.

The officers-elect, for the ensuing term are listed below:

HONORARY OFFICERS. *First past president* (1910-'12), ALFRED N. RICHARDS, professor of pharmacology at the University of Pennsylvania, secretary of the American Society of Biological Chemists and editor of the Journal of Biological Chemistry; *president*, P. B. HAWK, professor of physiological chemistry at Jefferson Medical College and author of "*Practical Physiological Chemistry*"; *vice-presidents*.—(1) HERMAN M. ADLER, instructor in psychiatry at the Harvard Medical School, pathologist and assistant physician in the Danvers State Hospital (Hathorne, Mass.); (2) ALLAN C. EUSTIS, assistant in internal medicine at Tulane University; (3) OLIVE G. PATTERSON, instructor in biological chemistry at Toronto University; WINIFRED J. ROBINSON, instructor in botany at Vassar College; LORANDE LOSS WOODRUFF, assistant professor of biology at Yale University.

ACTIVE OFFICERS. *President*, Walter H. Eddy; *vice-president*, Stanley R. Benedict; *secretary*, Alfred P. Lothrop; *treasurer*, William J. Gies. *Additional members of the executive committee*: Nellis B. Foster, Frederic G. Goodridge, Paul E. Howe. *Board of Directors of the BIOCHEMICAL BULLETIN*: Gustave M. Meyer, chairman, Charles F. Bolduan, Norman E. Ditman, C. Stuart Gager, A. J. Goldfarb, Louis Hussakof, Chester A. Mathewson, Oscar M. Schloss, Fred J. Seaver, and the secretary and treasurer of the association, *ex-officio*. *Additional members of the editorial*

committee: Ernest D. Clark, Nellis B. Foster, Frederic G. Goodridge, Paul E. Howe, Edgar G. Miller, Jr., Herman O. Mosenthal, Reuben Ottenberg, Jacob Rosenbloom, Emily C. Seaman, Clayton S. Smith.

William H. Welker, *Secretary*.

3. Columbia Biochemical Department

Death: On May 16, Thuisco Arthur Erpf-Lefkovich, B.S. (College of the City of New York, 1909), A.M. (in biological chemistry, Columbia, 1910). At the time of his decease, he was a second-year student of medicine and a candidate for the degree of Ph.D. in biological chemistry. His A.M. thesis was devoted to the results of an experimental study with Dr. Gies on the nature and diffusibility of preëxistent salins in protoplasm. During the past two years some of his time was given to embryo-chemical study, in collaboration with Drs. R. T. Frank and Jacob Rosenbloom. The untimely death of our young associate has deeply grieved a large body of warm friends.

Marriage: On June 25, Miss Florence Augusta Osborne and Dr. Alfred P. Lothrop.

Resignations and appointments. Last February, Dr. W. H. Welker resigned his assistant professorship, to take effect July 1. At the last regular meeting of the officers of the department, the following memorandum, presented by Professor Gies, was unanimously adopted:

The officers of the Biochemical Department of Columbia University have received, with deep personal and professional regret, the resignation of Prof. William H. Welker. During his successive terms as assistant (1904-1907), associate (1910-1911), and assistant professor (1911-1912) in this department, Dr. Welker has given the University devoted and efficient service, both as an executive officer and as a teacher. He has also engaged in research with earnestness and success.

Dr. Welker has performed his duties with enthusiasm and fidelity. He has been a genial and companionable associate. He has had the respect and esteem of all the students. He has made a permanent impress for good on the past as well as on the future of our department.

As he goes to another field of usefulness, Professor Welker takes with him the cordial good wishes of all his colleagues for his continuous success and happiness.

Dr. Paul E. Howe, instructor in physiological chemistry at the University of Illinois, has been appointed to the assistant professorship made vacant by Professor Welker's resignation.

Dr. Jacob Rosenbloom recently resigned his affiliated position at Mt. Sinai Hospital (page 362), to accept a similar relation to the German Hospital and Dispensary as assistant pathologist in charge of the chemical laboratory.

Dr. E. D. Clark, greatly to our regret, has resigned his instructorship to accept a similar position in Professor Benedict's department at Cornell Medical School made vacant by Dr. Fisher's retirement (page 570).

Dr. Harold E. Woodward has begun work as assistant chemist in the Food and Drug Inspection Laboratory of the U. S. Appraiser's Stores in Philadelphia.

Miscellaneous notes. Dr. Herman O. Mosenthal is spending a three months' leave of absence at the medical clinic in Tübingen. He is working there under the direction of Professors Romberg and Schlayer, observing especially their methods of treating cases of nephritis at the bedside and also investigating the action of diuretics in experimental animal nephritis. He will return about August 1.

At its last commencement Rutgers College conferred upon Clayton S. Smith the degree of M.S., in recognition of his teaching and research since his graduation from Rutgers in 1909.

Dr. John L. Kantor, who received both the M.D. and Ph.D. degrees at the last Columbia commencement, coöperated with Professor Gies in research during his vacations, from the time he became a medical student in 1908. The high quality of his work is indicated by the fact that he was one of the honor men in medicine, standing very nearly at the head of the "honor list."

Awards of higher degrees at Columbia to students of biological chemistry. *Doctors of philosophy.* Of the twenty-four recipients of the degree of Ph.D. under the Faculty of Pure Science, at Columbia's last commencement, thirteen had taken majors or minors, or both, in the biochemical department. The names of the

candidates, and their major and minor subjects are given below:¹

Name of Candidate	Major	Minor	Minor
David Alperin, M.D.	biological chemistry	bacteriology	organic chemistry
L. E. Bisch, M.D.	biological chemistry	medicine	medicine
J. J. Brönfenbrenner	biological chemistry	bacteriology	bacteriology
H. L. Fisher	organic chemistry	analytical chemistry	biological chemistry
M. L. Hamlin	organic chemistry	chemistry	biological chemistry
R. F. Hare	biological chemistry	biological chemistry	organic chemistry
J. D. Haseman	zoology	biological chemistry	physiology
Max Kahn, M.D.	biological chemistry	biological chemistry	organic chemistry
J. L. Kantor, M.D.	biological chemistry	medicine	medicine
C. A. Mathewson	biological chemistry	physiology	education
Miss W. J. Robinson	botany	biological chemistry	botany
Miss E. C. Seaman	biological chemistry	physiology	bacteriology
H. E. Woodward	physical chemistry	chemistry	biological chemistry

Masters of arts. The M.A. degree was recently conferred upon the following advanced students in the biochemical department: Edgar Altenburg; Donald B. Armstrong, Ph.B., M.D.; Elmer W. Baker, M.D.; Charles W. Ballard, Ph.C., Phar.D.; Ernst P. Boas, B.S.; Benjamin Horowitz, B.S.; Sidney Liebovitz; Louise McDanell, B.S.; Jessie A. Moore; Percy W. Punnett, B.S.; Abraham Ravich, M. D.; James B. Sidbury, M.D.

Doctor of pharmacy. Frank L. Hunt, Ph.G., Ph.C., one of the advanced students in biological chemistry at the School of Pharmacy received the degree of Phar.D.

Fellows: Walter P. Bliss, a student of biological chemistry, has held a "university fellowship" in bacteriology during the past year (see page 362).—A university fellowship in biological chemistry for 1912-13 has been awarded to Joseph S. Hepburn, B.S., University of Pennsylvania, 1906; M.S., 1907. At present Mr. Hepburn is one of Dr. Pennington's assistants in the U. S. Food Research Laboratory in Philadelphia.

Summer session. Courses in nutrition: page 518.

¹ Titles of the biochemical dissertations are given on page 546. Dr. Fred J. Seaver (Ph.D., Iowa State University, 1912) completed a minor in biological chemistry, in the Columbia biochemical department. The names of last year's candidates are summarized on page 150. Dr. Hare's name appears there and above. He passed his Ph.D. examinations in May, 1911, but continued his research at Columbia for some time thereafter. He presented printed copies of his dissertation last fall, when the degree was formally awarded (Oct. 17).

EDITORIALS

This volume has exceeded, by about 100 pages, the size originally contemplated. The Biochemical Association has received sufficient gifts from generous friends to enable it to meet all the

This volume expenses of publishing the first volume of the BULLETIN. The work of editing the volume has been exacting and continuous, but it has been a labor of love and a project of professional devotion.

It is our hope that the BULLETIN will steadily grow in the esteem of our colleagues and that it will merit the support of chemical biologists the world over. The expenses of the editorial office have been paid by the editors personally, who will continue indefinitely in this work without editorial salaries or allowances for expenses. An enlarging list of subscribers, and free labor in the editorial office, will enable us, in succeeding years, to lower the subscription price to a nominal sum and to expand the annual volume to 1000 pages or more. We intend to present, with increasing ability we trust, as much biochemical substance of as great variety and value, in as little space and for as little money, as possible. A large group of young, enthusiastic and industrious biological chemists is earnestly dedicated to this professional service.

Our cordial thanks are extended to each and every colleague of the many who have encouraged the establishment and are assisting in the maintenance of the BIOCHEMICAL BULLETIN. That much of **Appreciation, invitation, quotation** this assistance is an expression of personal friendship, and that it has little to do with professional expectations, is very clear to us. It is doubly appreciated on that account. But to our many colleagues whose faith in the professional utility of the BULLETIN is as strong as ours, and who have forwarded personal items and other contributions for its pages, we express not only the gratification we have already indicated by letter to each, but also the hope that their spirit of cooperation will become infectious.

We formally invite contributions to our editorial pages. We wish to make the Bulletin an open court for the presentation and consideration of any and all matters of interest to chemical biologists, and of influence on the advancement of biochemical science, at home and abroad.

We append a few quotations, from letters written by colleagues, which are related to this invitation:

1. "It struck me as not only odd but rather foolish to open an important *biochemical* journal with a portrait of a woman, a biography of a woman and a contribution by a woman. . . . I have since concluded to congratulate you on your serene indifference to the narrow view of those, like myself, who know that such considerations should have no influence in scientific affairs, but who cannot readily overcome the uncomfortable effects of long standing prejudice."

2. "Altho I believe Wiley is a born charlatan, as you know, I read with interest and, I am glad to add, with profit the editorial about him in the last number of the BULLETIN (page 523). I. O. N. (I'm 'on') evidently did his best, from an intimate (?) knowledge of the situation, to see all sides of the case and I think it is highly to your credit to afford opportunity for expression of opinions on such 'difficult' matters, whether you agree with them or not. . . . I am now considering the preparation of an exposure of current methods (in various biochemical and other types of laboratories) by which junior workers are shamefully robbed of credit due them by pirate chiefs who bend all their energies to self glorification. If you conclude to publish your editorial pages in an early issue *on asbestos sheets*, let me know and I'll forward a 'blast' on this theme that will do some good—or kill the BULLETIN."

3. . . . "But what I really wanted to send you this note for was to comment on the excellent spirit which, as editors, you put into the BULLETIN. You seem to have determined to make it a forum for open discussion, and without wishing to do anyone an injustice. So far as I know, only one other scientific editor of our acknowledged publications really does that. . . . This matter of open journals in the United States really strikes me as a far more important subject of discussion than any question in science itself."

4. "I understand that several very influential members of the American Biochemical Society (great admirers of Ben Zoate, too) consider that your editorial on a *professional code of ethics* (page 527) proves there is no need of or occasion for a biochemical journal

that is not devoted wholly to research. Get me? I had a warm conversation on the subject with . . . a few days ago and may send you a reflection of (and on) it for the September issue. I hope the BULLETIN will continue in professional opposition to all who feel that the American Biochemical Society 'has no business with ethics.'"

These quotations breathe a spirit of freedom, and show the devotion to high professional ideals, that we want the BIOCHEMICAL BULLETIN to embody and which, if our colleagues actively cooperate with us, will always animate it.

Occasionally we learn that some *narrow minded colleague* fails to understand how Johns Hopkins Hospital can have the effrontery to issue a *Bulletin* of international interest and value. He wants **Biochemical Bulletin:** to know, as he warms to the subject, why the **Why?** Marine Biological Laboratory at Wood's Hole, Mass., is so lost to decency as not only to maintain a *Biological Bulletin* of world wide acceptance, which is bad enough, but also to publish "local stuff" in it, which is worse than worse. Carried far beyond American shores by his merciless inquiry, he would like some one to explain, and that without any evasion, why Dr. B. Moore foisted his excellent *Bio-Chemical Journal*—one more—on the biochemical public and brazenly as well as successfully issues it "*From the Bio-Chemical Department, Johnston Laboratories, University of Liverpool.*" Our *narrow minded c.* demands more information on several such fibrillating subjects, altho the journals issued by Fake, Skinnem, and Graft, do not excite him. Last and certainly least, as his devastating glance sweeps the journalistic horizon, our *narrow m. c.* wants to be "shown" the justification for the feverish zeal and disquieting devotion of the Columbia University Biochemical Association—he is impatient to discover the disguised reason for its very evident purpose to be as useful as possible and to do, for the sheer love of it, all the work necessary for the establishment and continuance of the BIOCHEMICAL BULLETIN on a high plane of active service in the advancement of chemical biology and the promotion of biological chemistry as a useful and honorable profession. Why all this, he asks peremptorily. WHY! WHY!!

We are too busy and too agitated to undertake, now and here, to answer the important and disconcerting questions that relate to us—may the gods be merciful to Moore and the other suspects—but in a succeeding volume (subscribe, brother, subscribe) we may make the effort, provided, of course, that we can prevent foreclosure of the mortgage on our equipment and secure the assistance of a lawyer clever enough to construct a satisfying defense for us in this desperate emergency. The bell! Ah! A telegram! We look, perceive and smile. There it is, *n. m. c.*, take it, read it—and catch the idea, if your head is permeable to thoughts that do not vision self:

“Science is essentially mutualistic and *the success of one organization is the gratification of all*—the triumphs and discoveries of one are shared with the many *and the feeling of pride in the progress of the one may be shared without loss by sister organizations*. As the discovery made in one branch of science may be the necessary foundation for the solution of some problem in another, so the contribution from one society may be the stepping stone to advancement in another. It is all hail then, greetings and felicitation—and Godspeed in the accomplishments of your future destiny.” *Osborn*.

Students of fungous diseases of trees often desire to know what chemical changes are induced in wood by the activity of wood-destroying fungi. The literature dealing with this subject is unsatisfactory and scattering. The mere presence of a fungus on some trees causes staining and technical changes in the sound wood. Of the resultant chemical alterations we know nothing. The changes in strength are easy to measure. I shall be happy to facilitate work in these directions by furnishing material to any one desirous of studying problems connected with fungous diseases of trees.—*Homer D. House, Biltmore Forest School, Biltmore, N. C.*

“Clinical observation is the final court of appeal! This final court of appeal has been handing down its infallible decisions for thousands of years. They are received without question, until some obscure pathologist in the privacy of his laboratory examines them under the microscope,

**Significant medical
views**

and they are laughed out of court. Clinical observation teaches millions of people that they can cure all disease by dilutions of powder and sugar, millions more that they can cure all disease by tinkering with the spine, and millions more that they can cure all disease by denying its existence. It teaches one thing today and another tomorrow. *Clinical observation is not the final court of appeal!* It is an advocate before the bar, and when the advocate usurps the functions of the judge, and hands down decisions contrary to the evidence of sound pathology, we have a remedy—the recall.”—Ely.

“I hold that in the future, students who are being trained to be physiologists, whether in the field of physical and nervous or of chemical physiology, ought to have the M.D. degree. . . . Any doctor of philosophy who professes a different view I am compelled to regard as like the fox, in the fable, which lost its tail. If they, like this cunning animal, claim that a condition of taillessness is an advantage, I would warn all the young physiological foxes to beware. Grow a good bushy tail in the form of an M.D. after your name, and let no tailless old fox beguile you out of growing it.”—Henderson.

“Of great importance in connection with medical education in this country is a system by which specially trained medical teachers and research men in anatomy, physiology, pharmacology and pathology can be secured. At present it is very difficult to secure such teachers. . . . The lack of well-trained medical men has led many of our medical schools to fill positions with men holding the Ph.D., men well trained in their special sciences, but lacking the medical training, and, therefore [?], lacking the medical point of view. The lack of the medical point of view prevents such teachers from fully understanding the work of the clinical departments and correlating their work with those departments. Since there is so much to learn in a very limited space of time, the subjects of the medical course should be selected and taught by those who have received the complete medical training, and who are thereby better enabled to correlate these subjects with the other branches of the medical course.”—*Report of the Council on Medical Education, American*

Medical Association. (Journ. Amer. Med. Assn., lviii, p. 1796: June 8, 1912.)

Pasteur's discoveries were epoch-making, and revealed in him the Copernicus of medicine. Prior to his researches, the causes and rational treatment of disease were no better understood than in the stone age. Naturally, his conclusions were not accepted by medical men till every possible opposition had been exhausted. *Physicians resented instruction from a man devoid of medical training.* "A mere chemist" was the sneer most frequently on the lips of his adversaries.—*Martin.*

It would seem now as if the medical profession before 1868 was blindfolded and that its blindness was almost criminal; it is a sad record indeed, but we must look at it coolly *in order to understand what the auxiliary sciences can do for medicine.* Left to their own resources, practitioners during long centuries could do nothing against erysipelas and the other wound infections, but with the powerful aid of *bacteriology (which was founded by a chemist)*, surgery was able to triumph over these odious diseases and relegate them to the past.—*Editorial: Journal of the American Medical Association, 1912, lviii, p. 486.*

Does a protein *contain* amino acids or are the *radicals* only, of these acids, present in protein molecules? The comment of many writers on this and similar subjects, if accepted as good usage, would

Do proteins contain amino acids? justify the assertion that sodium chlorid is composed of hydrochloric acid and sodium or that potassium sulfocyanate contains prussic acid and potassium sulfid. Teachers of science should avoid slovenly phraseology. "Precision in presentation is on a par with perfection in demonstration."

In our first issue we alluded to a current tendency to misapply the term "body" to various substances such as purins (page 158). When our comment on this subject was written we did not know

Shall substances be called bodies? of the existence of the following request in the printed Directions for Assistant Editors and Abstractors of *Chemical Abstracts*: "Please do not use the word 'body' where 'substance' is intended."

The only way to learn to do great things is to do small things well, patiently, loyally.—*Jordan*.

The greatest joy of those who are steeped in work and who have succeeded in finding new truths and in understanding the relations of things to each other, lies in work itself.—*von Voit*.

Men are not young-hearted because they succeed; they succeed because they are young-hearted. The successful man is only a boy with a man's experience. America's most successful public man is also its biggest boy.—*Bili Rubin*.

The scientific temperament is in eternal conflict with the legal temperament. The one cares only for results; the other insists upon methods. The former is striving for something new; the latter sticks to precedents.—*Independent*.

Don't waste time patting yourself on the back. Don't get the fatal habit of believing a job is all right because you have done it. Don't let yourself believe that you are bigger than any one else in any particular line of human activity. This is a big world and there are a lot of very capable people in it. Above all things, don't admit publicly that you are more clever, honest or efficient than your neighbor. The siren that lures the average man to the rocks is the one that speaks with his own voice.—*Al K. Li*.

In fact men of science form, as it were, an organized army, laboring on behalf of the whole nation, and generally under its direction and at its expense, to augment the stock of such knowledge as may serve to promote industrial enterprise, to increase wealth, to adorn life, to improve political and social relations and to further the moral development of individual citizens. After the immediate practical results of their work we forbear to inquire; that we leave to the uninstructed. We are convinced that whatever contributes to the knowledge of the forces of nature or the powers of the human mind is worth cherishing, and may, in its own due time, bear practical fruits, very often where we should least have expected it.—*Helmholtz*.

INDEX

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(I) Author index (pp. 583-584);

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(A) *Impersonal subjects* (p. 584);

(B) *Personal subjects* (p. 592).

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The names of the authors of the leading papers, and the general subjects treated by each, may be obtained promptly from the summary of contents (pp. xi-xiv). This division of the index (I) includes not only the names of the main contributors, but also the names of accredited authors of abstracts, editorials, quotations, etc.

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COURSES OFFERED BY THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY. 1911-1912

Courses 51 and 105 are given during the first half year only. Course 101 is given during the first half year and is repeated (102) during the second half year. Courses 104 and 106 are given only during the second half year. All other courses are conducted throughout the entire academic year. All courses not otherwise specified are given at the College of Physicians and Surgeons.

(*Abbreviations:* C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51. ELEMENTARY ORGANIC CHEMISTRY. Introductory to courses 101, 102 and 104. (*Required of first year students of medicine.*) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, and Mr. Smith.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101-102. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (Teachers College, School of Household Arts.) L, 1 hr. R, 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Miss Seaman and Mr. Miller. (This course is designated "H. A. 25a" in the Teachers College Announcement.)

This course is designated "S—H. A. 25" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies and Miss Seaman.

104. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (*Required of first year students of medicine.*) L, 2 hr. R, 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, Dr. Clark, and Messrs. Smith and Rose.

This course is designated "S—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Mr. Smith.

106. GENERAL PATHOLOGICAL CHEMISTRY. *Lectures on nutrition in disease.* (Teachers College, School of Household Arts.) L, 1 hr. Prof. Gies. (This course, given this year for the first time, is designated "H. A. 25b" at Teachers College.)

201-202. CHEMISTRY OF NUTRITION. (School of Pharmacy. *Required of candidates for the Degree of Doctor of Pharmacy.*) L, 1 hr. Prof. Gies.

203-204. GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* L, 1 hr. Lw, 4 hr. Dr. Eddy.

Courses in Nutrition (continued)

205-206. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (Teachers College, School of Household Arts.) L, 1 hr. Lw, 5 hr. Prof. Gies and Miss Seaman. (This course is designated "H. A. 125" in the Teachers College Announcement.)

207-208. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS. L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Lothrop.

209-210. NUTRITION IN HEALTH AND DISEASE. L, 2 hr. Prof. Gies.

211-212. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies, Mr. Rose, and Dr. Clark.

213-214. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies.

215-216. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Welker, Drs. Lothrop and Clark and Mr. Rose.

TOXICOLOGY

217-218. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. Lw, 6 hr. Prof. Gies.

BOTANY

219-220. CHEMICAL PHYSIOLOGY OF PLANTS. (New York Botanical Garden.) L, 1 hr. Lw, 5 hr. Prof. Gies and Dr. Clark.

BACTERIOLOGY

221-222. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Clark.

SANITATION

105. SANITARY CHEMISTRY. (Teachers College, School of Household Arts.) L, 1 hr. Lw, 3 hr. Professor Gies, Miss Seaman and Dr. Clark. (This course is designated "H. A. 26, a" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301-302. BIOCHEMICAL SEMINAR. 1 hr. Prof. Gies.

RESEARCH IN BIOLOGICAL CHEMISTRY

Biochemical research may be conducted, by advanced workers, independently or under guidance. Prof. Gies consults regularly with investigators in the laboratories of Botany and Zoology on Tuesday afternoons, and at the N. Y. Botanical Garden on Wednesday afternoons.

LABORATORIES FOR ADVANCED WORK IN BIOCHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College and the New York Botanical Garden. The laboratories are well equipped for research in nutrition and all other phases of biological chemistry.

BIOCHEMICAL LIBRARY

Prof. Gies' library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons and is accessible, by appointment, to all workers in the Department.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

The Biochemical Association holds quarterly scientific meetings, which are open to all students in the University.

SUMMER SCHOOL COURSES

Summer session courses are mentioned in the foregoing references to Courses 101-102 and 104. Prof. Gies will have charge of both courses this summer. He will also conduct a special lecture course in nutrition. The laboratories will be open for research throughout the summer.



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WILLIAM H. WELKER, A.C., Ph.D., <i>Assistant Professor</i> .	REUBEN OTTENBERG, M.D., <i>Assistant</i> .
NELLIS B. FOSTER, M.D., <i>Associate</i> .	ANTON R. ROSE, A.M., <i>Assistant</i> .
WALTER H. EDDY, A.M., Ph.D., <i>Associate</i> . Secretary of the Staff.	CLAYTON S. SMITH, B.S., <i>Assistant</i> .
JACOB ROSENBLUM, M.D., Ph.D., <i>Associate</i> .	EDGAR G. MILLER, JR., A.B., <i>Assistant</i> .
ALFRED P. LOTHROP, A.M., Ph.D., <i>Instructor</i> . Department Registrar.	CHRISTIAN SEIFERT, <i>Laboratory Ass't</i> .
HERMAN O. MOSENTHAL, M.D., <i>Instructor</i> .	STELLA WALDECK, <i>Recorder</i> .
EMILY C. SEAMAN, A.M., <i>Instructor</i> .	CONSTANCE C. HART, <i>Laboratory Assistant</i> .
	BLANCHE R. HARRIS, <i>Laboratory Assistant</i> .
	M. V. MILLER, A.B., <i>Laboratory Assistant</i> .

COURSES OFFERED BY THE BIOCHEMICAL DEPARTMENT OF COLUMBIA UNIVERSITY. 1911-1912

Courses 51 and 105 are given during the first half year only. Course 101 is given during the first half year and is repeated (102) during the second half year. Courses 104 and 106 are given only during the second half year. All other courses are conducted throughout the entire academic year. All courses not otherwise specified are given at the College of Physicians and Surgeons.

(Abbreviations: C, conference; D, demonstration; L, lecture; Lw, laboratory work; R, recitation.)

ORGANIC CHEMISTRY

51. ELEMENTARY ORGANIC CHEMISTRY. Introductory to courses 101, 102 and 104. (Required of first year students of medicine.) L, 1 hr. D, 1 hr. R, 2 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, and Mr. Smith.

NUTRITION (PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY)

101-102. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (Teachers College, School of Household Arts.) L, 1 hr. R, 1 hr., each section (2). Lw, 5 hr., each section (2). Prof. Gies, Miss Seaman and Mr. Miller. (This course is designated "H. A. 25a" in the Teachers College Announcement.)

This course is designated "S—H. A. 25" in the Teachers College Division of the Summer School Announcement. The course was given last summer by Prof. Gies and Miss Seaman.

104. GENERAL PHYSIOLOGICAL CHEMISTRY. *A course in the elements of normal nutrition.* (Required of first year students of medicine.) L, 2 hr. R, 1 hr., each section (2). Lw, 6 hr., each section (2). Profs. Gies and Welker, Dr. Clark, and Messrs. Smith and Rose.

This course is designated "S—104" in the Medical Division of the Summer School Announcement. It was given last summer by Prof. Gies and Mr. Smith.

106. GENERAL PATHOLOGICAL CHEMISTRY. *Lectures on nutrition in disease.* (Teachers College, School of Household Arts.) L, 1 hr. Prof. Gies. (This course, given this year for the first time, is designated "H. A. 25b" at Teachers College.)

201-202. CHEMISTRY OF NUTRITION. (School of Pharmacy. Required of candidates for the Degree of Doctor of Pharmacy.) L, 1 hr. Prof. Gies.

203-204. GENERAL BIOLOGICAL CHEMISTRY. *Specially adapted to the needs of secondary school teachers of biology.* L, 1 hr. Lw, 4 hr. Dr. Eddy.

Courses in Nutrition (continued)

205-206. ADVANCED PHYSIOLOGICAL CHEMISTRY, INCLUDING METHODS OF RESEARCH IN NUTRITION. (Teachers College, School of Household Arts.) L, 1 hr. Lw, 5 hr. Prof. Gies and Miss Seaman. (This course is designated "H. A. 125" in the Teachers College Announcement.)

207-208. BIOCHEMICAL METHODS OF RESEARCH, INCLUDING CLINICAL METHODS. L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Lothrop.

209-210. NUTRITION IN HEALTH AND DISEASE. L, 2 hr. Prof. Gies.

211-212. NUTRITION IN HEALTH. *A laboratory course in advanced physiological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies, Mr. Rose, and Dr. Clark.

213-214. NUTRITION IN DISEASE. *A laboratory course in advanced pathological chemistry.* L, 2 hr. Lw, 14 hr. Prof. Gies.

215-216. ADVANCED PHYSIOLOGICAL AND PATHOLOGICAL CHEMISTRY, INCLUDING ALL PHASES OF NUTRITION. Research. C, 1 hr. (individual students). Lw, 16 hr. Profs. Gies and Welker, Drs. Lothrop and Clark and Mr. Rose.

TOXICOLOGY

217-218. EFFECTS AND DETECTION OF POISONS, INCLUDING FOOD PRESERVATIVES AND ADULTERANTS. Lw, 6 hr. Prof. Gies.

BOTANY

219-220. CHEMICAL PHYSIOLOGY OF PLANTS. (New York Botanical Garden.) L, 1 hr. Lw, 5 hr. Prof. Gies and Dr. Clark.

BACTERIOLOGY

221-222. CHEMISTRY OF MICROORGANISMS: FERMENTATIONS, PUTREFACTIONS AND THE BEHAVIOR OF ENZYMES. *An introduction to sanitary chemistry.* L, 1 hr. Lw, 7 hr. Prof. Gies and Dr. Clark.

SANITATION

105. SANITARY CHEMISTRY. (Teachers College, School of Household Arts). L, 1 hr. Lw, 3 hr. Professor Gies, Miss Seaman and Dr. Clark. (This course is designated "H. A. 26, a" in the Teachers College Announcement.)

BIOCHEMICAL SEMINAR

301-302. BIOCHEMICAL SEMINAR. 1 hr. Prof. Gies.

RESEARCH IN BIOLOGICAL CHEMISTRY

Biochemical research may be conducted, by advanced workers, independently or under guidance. Prof. Gies consults regularly with investigators in the laboratories of Botany and Zoology on Tuesday afternoons, and at the N. Y. Botanical Garden on Wednesday afternoons.

LABORATORIES FOR ADVANCED WORK IN BIOCHEMISTRY

The laboratories in which the advanced work of the biochemical department is conducted are situated at the College of Physicians and Surgeons, Teachers College and the New York Botanical Garden. The laboratories are well equipped for research in nutrition and all other phases of biological chemistry.

BIOCHEMICAL LIBRARY

Prof. Gies' library occupies a room adjoining the main biochemical laboratory at the College of Physicians and Surgeons and is accessible, by appointment, to all workers in the Department.

COLUMBIA UNIVERSITY BIOCHEMICAL ASSOCIATION

The Biochemical Association holds quarterly scientific meetings, which are open to all students in the University.

SUMMER SCHOOL COURSES

Summer session courses are mentioned in the foregoing references to Courses 101-102 and 104. Prof. Gies will have charge of both courses this summer. He will also conduct a special lecture course in nutrition. The laboratories will be open for research throughout the summer.

New York Botanical Garden Library



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